LEVEL II

(11)

(6)

PROBLEM DEFINITION STUDY ON TAX (1-ACETYLHEXAHYDRO-3,5-DINITRO-1,3,5-TRIAZINE), SEX (1-ACETYLOCTAHYDRO-3,5,7-TRINITRO-1,3,5,7-TETRAZOCINE), LEAD SALICYLATE AND LEAD β-RESORCYLATE 2-NITRODIPHENYLAMINE AND ETHYL CENTRALITE.

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(10) R.S. Wentsel, M.J. Wilkinson, W.H. Fitzpatrick, W.E. Harward, III, W.E. Jones, III, J.F. Kitchens

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1-acetylhexahydro-3,5-dinitro-1,3,5-triazine (TAX), 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX), lead salicylate, lead 8-resorcylate, 2-nitrodiphenylamine, ethyl centralite, problem definition study, review, manufacture use, toxicology, chemistry, physical properties, environmental fate

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
The objective of this study was to evaluate the available information on the toxicological and environmental hazards associated with Army munitions production. The chemicals included in this study were TAX, SEX, lead salicylate, lead 8-resorcylate, 2-nitrodiphenylamine, ethyl centralite. Recommendations for further studies to elucidate the environmental fate and toxicological properties of these compounds are made to fill the information gaps identified in the literature review.

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Final Report

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W.H. Fitzpatrick W.E. Harward, III
W.E. Jones, III J.F. Kitchens

July 1979

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Contract No. DAMD17-77-C-7057

EXECUTIVE SUMMARY

The goal of this problem definition study was to evaluate the literature relating to the toxicological and environmental hazards associated with Army use or pollution of six (6) chemicals associated with munitions production. The chemicals included in this study were:

TAX (1-acetylhexahydro-3,5-dinitro-1,3,5-triazine)

SEX (1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine)

Lead β-Resorcylate

Lead Salicylate

2-Nitrodiphenylamine

Ethyl Centralite

The results of this study will help the Army establish research priorities and aid in recommending effluent criteria for these compounds. The recommendations resulting from this study are discussed below:

SEX and TAX

SEX (1-acetylhexahydro-3,5-dinitro-1,3,5-triazine) and TAX (1-acetyl-octahydro-3,5,7-trinitro-1,3,5,7-tetrazocine) are byproducts of the manufacture of the explosives RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) and HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine). The explosives are manufactured by the Army at Holston Army Ammunition Plant in Kingsport, Tennessee. Current discharges of SEX and TAX to the Holston River are estimated at 14,000-28,000 and 42,000-63,000 lbs/year, respectively.

Information on the physical and chemical properties, biological interactions and environmental fate of SEX and TAX is very limited. Thus, the properties of these compounds must be inferred from known properties of related compounds such as RDX and HMX.

In view of the limited amount of information available on SEX and TAX, the following studies are recommended in order to fill in information gaps:

- definition of the solubility and reactivity of SEX and TAX in aqueous solutions
- verification of discharged data from Holston AAP
- acute mammalian toxicological studies
- bioaccumulation and biodegradation studies
- acute and chronic aquatic toxicity studies

Lead β-Resorcylate and Lead Salicylate

Lead β-resorcylate and lead salicylate are used as burning rate modifiers in solid propellant formulations. The environmental fate and toxicological properties of these compounds are unknown or inferred from experimental data on lead acetate. These compounds are currently used only at Radford AAP although Badger and Sunflower AAP's would also use lead salicylate when they are operational.

In order to provide more complete information on the toxicity and environmental fate of these compounds, the following studies are recommended:

Lead β-Resorcylate

- further enumeration of the composition, chemistry, and analysis methods for salts
- sampling and analysis at Radford AAP to determine amounts of lead β-resorcylate in the effluent and accumulation in the New River sediment and biota
- acute mammalian toxicity study
- chronic mammalian toxicity study
- acute and chronic aquatic toxicity tests with fish and invertebrates
- determine the effectiveness of proposed treatment facilities to remove lead β-resorcylate from Radford's AAP effluents

Lead Salicylate

- further investigation of the physical and chemical properties of lead salicylate
- chronic feeding study to determine the long-term effects of exposure to lead salicylate
- an acute skin LD50 to determine skin adsorption
- aquatic toxicity studies with fish and invertebrates

2-Nitrodiphenylamine

2-Nitrodiphenylamine is a stabilizer used by the Army in the manufacture of solid propellants. Currently, Radford AAP is the only Army Ammunition Plant producing propellants containing 2-nitrodiphenylamine. This compound enters the environment in the wastewater from the nitroglycerin manufacture and propellant formulation areas. The amount entering the New River during full mobilization operations at Radford AAP is estimated at 80-200 lb/month.

2-Nitrodiphenylamine has a low acute toxicity to mammals. Metabolism to 4-hydroxy- and 4,4'-dihydroxy-2-nitrodiphenylamine and rapid elimination from the body, similar to that observed for diphenylamine is likely. N-hydroxyl-2-nitrodiphenylamine is also postulated as a metabolite based on the appearance of methemoglobin in the blood.

The following studies are recommended in order to obtain the needed information to fully determine the environmental hazards of 2-nitrodiphenylamine in the Radford AAP effluent:

- studies to determine the water solubility and octanol/water partition coefficient of 2-nitrodiphenylamine
- sampling and analysis of Radford AAP's effluent for 2-nitrodiphenylamine and correlation of effluent concentrations with production data
- biodegradation studies on 2-nitrodiphenylamine
- detailed aquatic toxicity studies on 2-nitrodiphenylamine

Ethyl Centralite

Ethyl centralite is used as a stabilizer in solid propellant formulations. Currently, only Radford AAP is using this compound. Most of the ethyl centralite is imported from Europe, however, Van De Marck Chemical Co. is now making ethyl centralite under contract to the Army.

In acute exposure, ethyl centralite has a low toxicity to mammals by the oral, inhalation or cutaneous routes. During chronic exposure to this chemical, only subtle biochemical changes are observed. The evidence to date suggests that this compound is not mutagenic or carcinogenic. There is no information on the teratogenic potential of ethyl centralite.

The environmental fate of ethyl centralite is not known. It is toxic to aquatic organisms in the low ppm levels. From the data available, it appears that ethyl centralite is persistent in the environment and probably bioconcentrates in aquatic organisms.

The following studies are recommended based on the potential environmental hazards of ethyl centralite:

- sampling and analysis of Radford AAP effluent, the New River, sediment and biota
- further studies on the physical and chemical properties of this compound
- microbial degradation studies
- assessment of the ability of the proposed treatment facilities at Radford AAP to efficiently remove ethyl centralite from the effluent.

FOREWORD

The purpose of this study was to gather, collate and evaluate the available information on the toxicological and environmental hazards of TAX, SEX, lead β-resorcylate, lead salicylate, 2-nitrodiphenylamine and ethyl centralite. The results of this study will aid the Army in establishing future research needs and in recommending effluent criteria for these compounds.

In the preparation of this report, several reference sources have been directly quoted. Permission has been obtained from the appropriate sources for reprint of the quoted information.

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PROBLEM DEFINITION STUDY ON
TAX
(1-ACETYLHEXAHYDRO-3,5-DINITRO-1,3,5-TRIAZINE)

SUMMARY

TAX (1-acetylhexahydro-3,5-dinitro-1,3,5-triazine) is a by-product of RDX/HMX manufacture. The only current United States manufacturer of RDX and HMX is Holston Army Ammunition Plant, located in Kingsport, Tennessee. TAX is present in the wastewaters from Holston AAP at levels ranging from 60 to 90% of the RDX content. Based on these ratios, 42,000 to 63,000 lb/year of TAX are expected to be discharged under current operating levels. Estimates of the quantity of TAX discharged are between 1.3 and 1.9 million lb/year at full mobilization production of RDX and HMX.

Only limited information is available on the physical and chemical properties of TAX. No information is available on the toxicological or environmental properties of this compound. Thus, the toxicological or environmental hazards of this compound must be inferred from the available information on RDX. RDX has an i.v. LD50 of 18.7 mg/kg in mice. Oral LD50's range from 100 mg/kg to 500 mg/kg depending on the physical form of the RDX. RDX is moderately toxic to fish with LC50's in the 4 to 6.5 ppm range. Sensitivity of aquatic invertebrates to RDX varies. The midge is reportedly sensitive to concentrations above 1.3 ppm while *Daphnia magna* is not sensitive to 20 ppm. The presence of the $\geq N-\overset{O}{\overset{\|}{C}}-CH_3$ group in TAX is expected to increase its biological activity over that of RDX. However, the extent to which the presence of the functional group will alter the toxicological properties of TAX over those of RDX is not predictable.

In view of the limited information on TAX, the following studies are recommended in order to fill in information gaps:

- further definition of the solubility limits and reactivity of TAX in aqueous solutions
- additional sampling and analysis at Holston AAP to verify previous data
- an acute mammalian toxicological study
- bioaccumulation and biodegradation studies

FOREWORD

A. Study Goals

This report presents the results of an evaluation of the available information on the toxicological and environmental hazards of TAX (1-acetyl-hexahydro-3,5-dinitro-1,3,5-triazine). TAX is a by-product of RDX and HMX manufacture at Holston AAP. This compound is found in the wastewaters from the manufacturing process. Holston AAP is the only source from which entry of TAX into the environment occurs in the United States. This evaluation of the toxicological and environmental hazard of TAX was undertaken in order to aid the Army in identification of research needs and in recommendations of environmental criteria for this compound.

B. Study Methodology

The methodology utilized to gather information for this report included a detailed search of the literature and numerous personal contacts. During the literature search, the following sources were reviewed for pertinent information on TAX:

- Chemical Abstracts	1940 - present
- Biological Abstracts	1950 - present
- Excerpta Medica	1950 - present
- TOXLINE	1965 - present
- National Technical Information Service	1964 - present
- Defense Documentation Center	1958 - present
- COMPENDEX	1970 - present

The search of the literature revealed very little information on TAX.

Personal contacts were made with Army Ammunition Plant personnel and Army and civilian researchers. The specific contacts made and results are presented below:

1. Manufacturers of RDX

a. Holston AAP Personal Contacts

Mr. Harold Shell and Captain Morris were contacted October 2, 1978 for information on TAX. They found no toxicological or health effects data in their files. A trip was made to Holston AAP on January 17, 1979. We met with Mr. Bob Hash, Mr. Sam Wright and Mr. Buck Rogers. References on the chemical properties of TAX were received and we were told of recent research on TAX. Mr. Bob Hash indicated that some recent effluent data on TAX was collected. On January 18, 1979, a letter was written to Mr. Mike Mills requesting the effluent data and other information. The available information was received February 26, 1979.

b. Foreign

Only one manufacturer of RDX was listed in the 1978 SRI Chemical Producers Directory of Western Europe. PRBsa in Belgium was contacted and supplied references for toxicological studies concerning RDX and HMX.

2. Other Sources

Dr. Jay Abercrombie of the U.S. Army Chemical Systems Laboratory, Aberdeen Proving Ground, MD was contacted September 12, 1978. He reported no information on TAX. Mr. J. Gareth Pearson of AMRDC, Fort Detrick, Md. was visited in September 1978. He had no toxicity data on TAX.

Dr. David Liu of SRI International was contacted about his current study on RDX. We received monthly reports and mammalian toxicity data on RDX.

Mr. R. Bentley of EG & G Bionomics was contacted for aquatic toxicity data on RDX.

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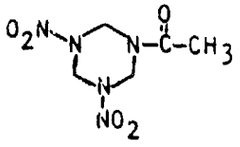
I. TAX (1-acetylhexahydro-3,5-dinitro-1,3,5-triazine)

A. Alternate Names

TAX (1-acetylhexahydro-3,5-dinitro-1,3,5-triazine), a cylic nitramine formed as a by-product in the manufacture of RDX, has a molecular formula of $C_5H_9N_5O_5$, a molecular weight of 219.15 and the following structural formula.



Pertinent alternate names for TAX are listed below:

CAS Registry No.:	14168-42-4
C.A. Name (9CI):	1,3,5-triazine, 1-acetylhexahydro-3,5-dinitro-
C.A. Name (5CI):	s-triazine, 1-acetylhexahydro-3,5-dinitro-
Wiswesser Line Notation:	T6NVI CNNW ENNWTJ
Synonyms:	TAX; 1-acetyl-3,5-dinitro-1,3,5-triaza-cyclohexane

B. Physical and Chemical Properties

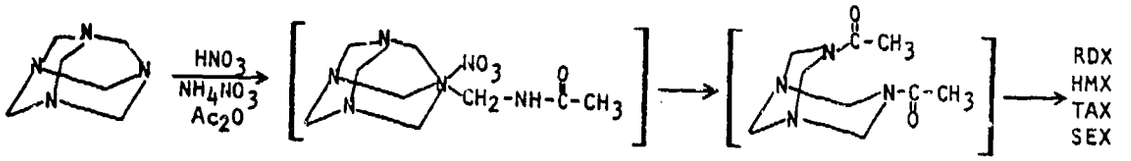
1. Physical Properties

Available physical properties of TAX are listed in Table I-1. The ultraviolet absorption spectrum of TAX is shown in Figure I-1.

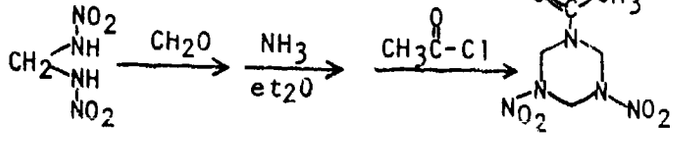
2. Chemical Properties

a. General Chemistry

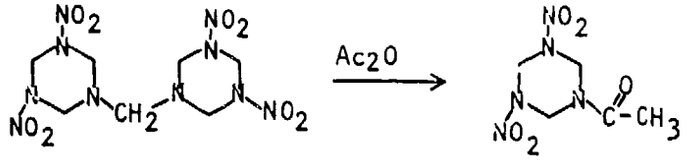
TAX is obtained as a by-product in the synthesis of RDX and HMX from hexamine by the Bachmann and Sheehan (1949) procedure. Aristoff *et al.* (1949) suggested a number of likely intermediates in this reaction which could explain the formation of TAX as well as RDX, HMX and SEX.



TAX has also been synthesized by several other routes. Chapman *et al.* (1949) synthesized TAX from methylene dinitroamine and formaldehyde:



TAX has been synthesized from methylene-bis(3,5-dinitro-1,3,5-triazine) Dunning and Dunning, 1950a),



from a number of substituted 1-methyl-3,5-dinitro-1,3,5-triazines (Dunning and Dunning, 1950b)

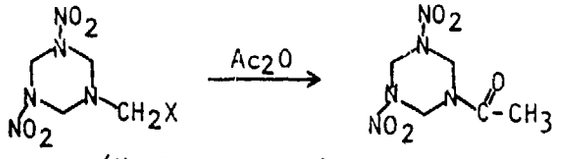


Table I-1. Physical Properties of TAX*

Physical Form @ 20°C:	solid, prismatic plates
Color:	colorless
Melting Point:	156 - 158°C
Volatility:	no data available
Vapor Pressure:	no data available
Octanol-Water Partition Coefficient:	no data available
Solubility:	water soluble in the ppm level; in general, slightly more soluble than RDX in various solvents
IR (KBr):	3060, 1660, 1580, 1420, 1370, 1280, 1240, 1180, 1030, 990, 920, 880, 850, 810, 750, 630, 585, 490 cm^{-1}
NMR:	δ 6.25 (singlet, 2H, $(O_2NN)_2-CH_2$) δ 5.80 (singlet, 4H, $(O_2NN)-CH_2-NCO-$) δ 2.27 (singlet, 3H, $-CH_3$)

*Dunning and Dunning, 1950 a,b,c; Chapman *et al.*, 1949; Aristoff *et al.*, 1949; Gilbert *et al.*, 1976.

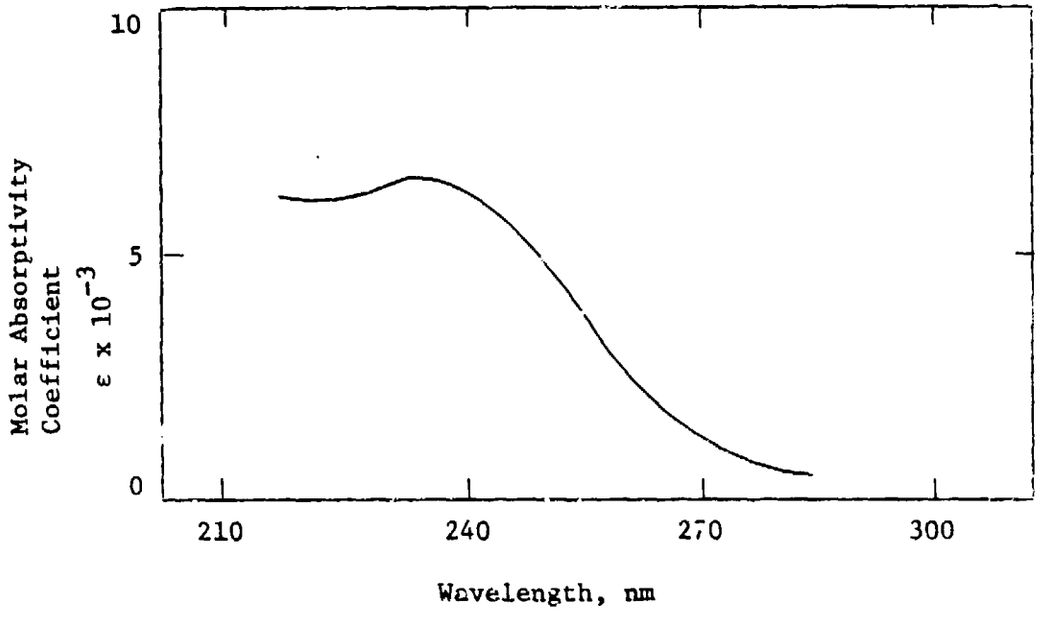
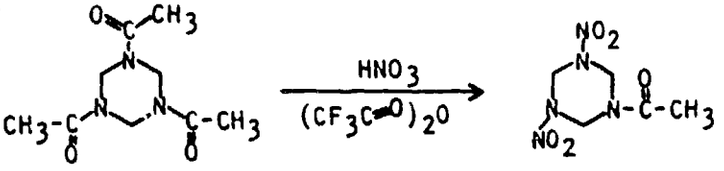
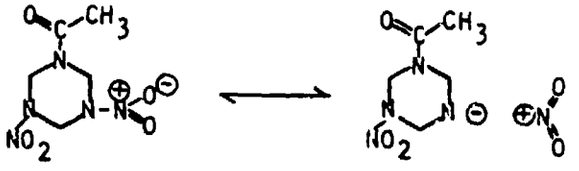


Figure I-1. Ultraviolet Spectrum of TAX (Schroeder *et al.*, 1951)

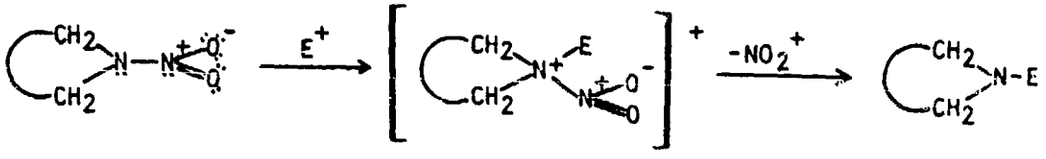
and from tri-N-acyl triazines (Dunning and Dunning, 1950c).



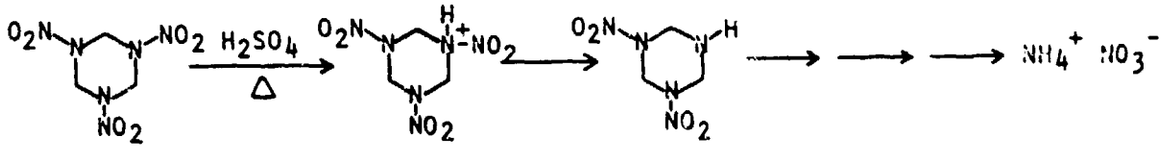
The reactions of TAX, as those of nitramines in general, are greatly influenced by the partial ionic character of the nitrogen-nitrogen bonds (Stals, 1969).



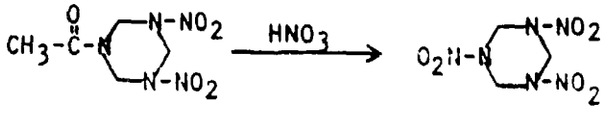
The partially negative nitrogen is subject to electrophilic substitution which can be generalized by the following scheme:



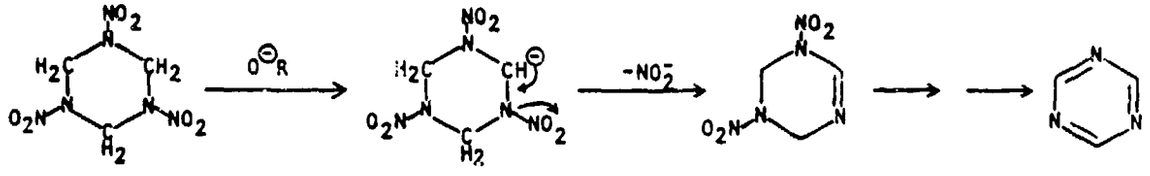
The acid decomposition of RDX is a specific example of electrophilic substitution (Stals, 1969).



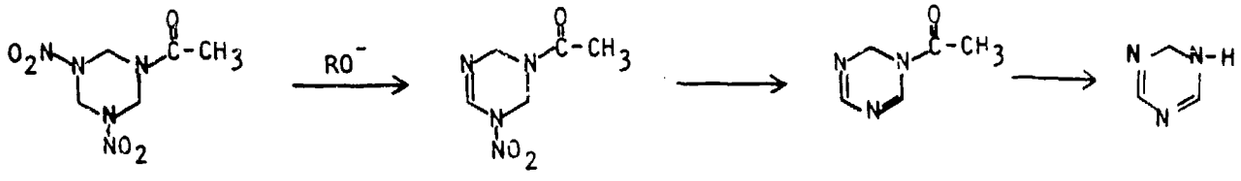
The acid decomposition of TAX has been reported to yield 3 moles of formaldehyde and 1 mole of ammonia (Aristoff *et al.*, 1949), acetic acid and either nitrogen gas or nitrous oxide are most likely formed as well. TAX can be converted to RDX by reaction with nitric acid:



Secondary nitramines are attacked by strong base resulting in elimination reactions. RDX, for example, reacts with alkoxides yielding triazines (Stals, 1969):



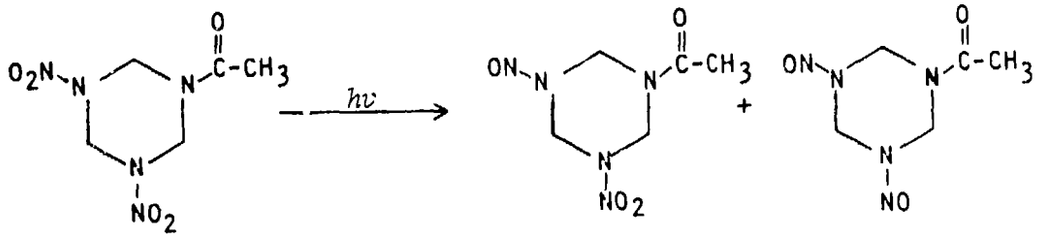
An analogous reaction for TAX would most likely involve two similar base catalyzed elimination steps and a nucleophilic displacement of the amide function leading to dehydrotriazine.



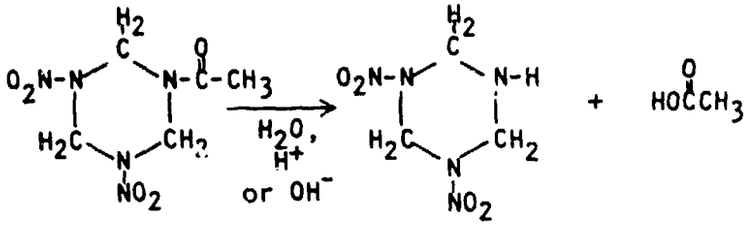
b. Environmental Reactions

The environmental fate of TAX has not been determined. However, recent studies on the environmental fate of RDX have been conducted. Photolysis of RDX has been carried out with both artificial irradiation and sunlight. Under artificial conditions, the mononitroso derivative of RDX was shown to be the major photoproduct (Kubose and Hoffsommer, 1977; Sikka *et al.*, 1978). Nitrate, nitrite, formaldehyde and nitrogen are also formed. The extent of photolysis to the mononitroso derivative is dependent on pH and nitrite ion concentration. Low pH and high nitrite concentrations inhibit the initial photolysis. Dissolved oxygen has no effect on the photochemistry. The quantum yields for RDX photolysis in a pH 8.08 borate buffer is 0.69 (Sikka *et al.*, 1978). Photolysis of RDX in tap water in natural sunlight also occurs. The half life for sunlight photolysis of RDX is estimated at 9-13 hours (Sikka *et al.*, 1978).

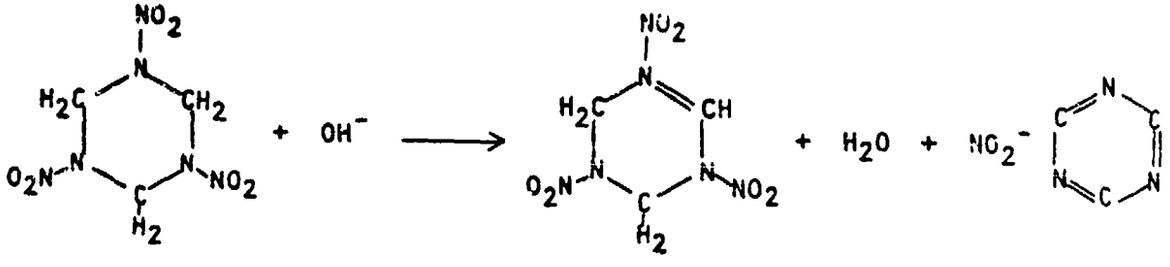
A similar photolysis reaction for TAX would be expected yielding the following products:



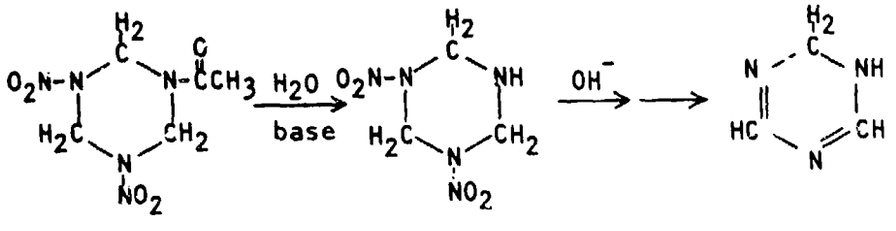
Hydrolysis of TAX in the environment is also likely if conditions are severe enough.



Although the hydrolysis of TAX has not been studied, a "hydrolysis" reaction has been reported for RDX. At pH 2.7 virtually no hydrolysis occurs within 12 days (Sikka *et al.*, 1978). At pH 9.07 and 31°C, 27% of the RDX is hydrolyzed in 21 days. In studies of basic hydrolysis of RDX, Hoffsommer *et al.* (1977) show that the reaction is a concerted E2 elimination process with proton abstraction by hydroxide ion and simultaneous loss of nitrate.



A similar reaction could occur for TAX. As acetyl is a poor leaving group the most likely product would be the corresponding dihydrotriazine.



C. Monitoring and Analysis

1. Analytical Methods

Analysis for TAX has been reported by a semi-quantitative column chromatography technique (Malmberg *et al.*, 1953). TAX was chromatographed on silicic acid/Celite 535 (2:1). Partial separations (TAX was not completely separated from SEX) (1-acetyloctahydro-1,3,5-trinitro-1,3,5,7-tetrazocine) were obtained using 50% ethyl acetate in benzene as a mobile phase. Quantitative removal of TAX from the adsorbent was about 91% efficient using a 1:1 acetone-ether solution. The colorless chromatographic zones were visualized using streak reagents which consisted of zinc dust, benzene, sulfanilic acid and 1-naphthylamine in acetic acid on the extruded column. A deep red-pink color afforded detection of as little as 0.01 mg of the nitramine.

Bell and Dunstan (1966) have studied the use of thin layer chromatography for the analysis of TAX and other nitramines. Chromatograms were run on Silica gel G using benzene/nitromethane, chloroform/nitromethane and petroleum ether/acetone solvent system. Visualization was accomplished by spraying with diphenylamine and irradiating with ultraviolet light.

Analysis by high performance liquid chromatography has also been accomplished (Holston Defense Corporation, 1977). Chromatograms were run using a Li Chrosorb Si 60 column in isocratic mode using a methanol, acetonitrile, chloroform, iso-octane (1:2:3:14) solvent system. Detection was accomplished by a 245 nm ultaviolet detector. Lowest concentration detected was 0.001 mg/l.

2. Monitoring

In 1977, Holston Defense Corporation sampled three effluent streams and one point in the Holston River for the presence of TAX and other related compounds. The sampling locations were as follows:

- N-3 - manhole below Building N-3 which carries effluents from the G (RDX recrystallization), H, I, J, K, M and N (RDX, HMX incorporation) Buildings on lines 1 through 5.

- N-6 - manhole below Building N-6 which carries process effluents from Buildings D6 (nitration), E6 (acids removal and explosives wash) and G6 (recrystallization).

- T-2 - manhole below T-2 (acid area) which carries process effluents from Buildings C3, C5 (reagent preparation), B9, B11 (primary distillation and ammonia recovery), D3, D5 (nitration), E3 and E4 (acids removal and explosives wash).

- Holston River at the area B Boundary

The results of the samples analysed for TAX and other related compounds are presented in Table I-2. Sample analyses for TAX varied considerably with the type of storage, therefore for consistency, only those samples which were extracted and stored in acetonitrile were reported.

From the data in the table, it appears that TAX is entering the environment from the nitration buildings (D-buildings), the acids removal and explosives wash (E-Buildings), recrystallization (G-Buildings), dewatering and incorporation steps.

Average concentrations of RDX, TAX and HMX at the four sample points are given below.

Sample Point	RDX(mg/l)	TAX(mg/l)	HMX(mg/l)
N-3	5.5	4.8	2.8
N-6	4.5	2.6	1.6
T-2	0.3	0.02	0.12
River	0.01	0.004	0.01

In the effluent from the dewatering and incorporation steps, TAX is present at levels of between 60 and 90% of the RDX content. At full mobilization ∿ 208 million pounds of RDX would be produced each year. If 1% of this amount is lost in the effluents, then Holston AAP could discharge as much as 2.1 million pounds of RDX per year. Discharges of TAX could be 1.3 to 1.9 million pounds per year if the 60-90% ratio of TAX/RDX is valid.

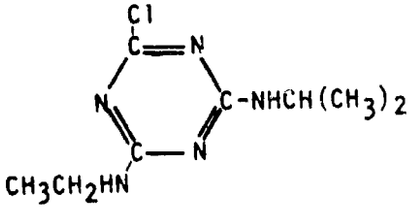
Table I-2. Analysis of Waste Streams for RDX, HMX, TAX and SEX (Holston Defense Corporation, 1977).

Date	Sample Point	RDX (mg/l)	TAX (mg/l)	HMX (mg/l)	SEX (mg/l)
5/17-18/77	N-3	10.0	8.7	2.2	2.2
	N-6	6.3	6.3	1.3	0.2
	T-2	0.2	*	0.04	*
	River	0.01	0.003	*	*
5/24-25/77	N-3	7.2	16.9	2.4	4.0
	N-6	2.2	2.2	1.3	1.0
	T-2	0.4	*	0.6	*
	River	0.007	0.003	0.03	0.001
5/31/77-6/1/77	N-3	2.4	*	4.5	4.8
	N-6	7.0	3.1	2.1	0.6
	T-2	0.4	*	0.07	*
	River	0.006	*	0.006	*
6/6-7/77	N-3	4.3	*	1.5	1.9
	N-6	5.6	5.6	1.7	1.5
	T-2	0.4	0.007	0.1	0.001
	River	0.009	0.003	0.006	0.0007
6/8-9/77	N-3	2.1	2.2	0.3	0.8
	N-6	1.0	1.0	0.4	0.3
	T-2	0.1	0.005	0.03	*
	River	0.0005	0.01	0.0009	*
6/13-14/77	N-3	10.4	5.8	2.9	2.2
	N-6	3.9	0.007	2.4	1.3
	T-2	0.2	0.04	*	*
	River	0.02	0.001	0.01	*
6/17-19/77	N-3	1.8	0.007	4.7	0.5
	N-6	5.3	3.4	2.3	0.07
	T-2	0.3	0.1	*	*
	River	0.02	0.005	0.03	*

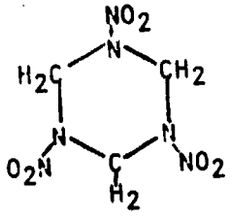
*not detected

D. Health Effects

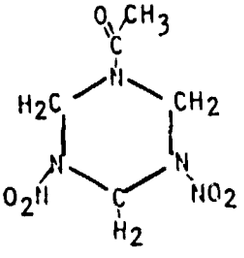
No information on the toxicological properties of TAX was found in the literature or through personal contacts. Therefore, the toxicology of TAX must be inferred from the information available on related compounds. The basic s-triazine structure is found in a variety of compounds including many pesticides. However, the triazine pesticides are usually substituted on the carbons and not the nitrogen. For example, the pesticide, atrazine, has the following formula:



The substitution on the nitrogen will change the chemical and toxicological properties of TAX from those observed with the pesticides. Thus, TAX is expected to behave more like RDX than the triazine pesticides.



RDX



TAX

The presence of the $>N-\overset{O}{\overset{\|}{C}}-CH_3$ is expected to increase the biological activity of TAX from that observed with RDX. The extent of this increase in biological activity is not predictable.

The material presented, herein, is based on toxicological studies of RDX. These data are related to TAX in that the gross toxicological effects of these two compounds are expected to be similar. However, the degree of the effect of TAX will be altered somewhat from that of RDX.

1. Biology

a. Absorption

Absorption of RDX and presumably TAX occurs through the gastrointestinal tract and respiratory tract. No information is available on the efficiency of absorption of RDX by the respiratory tract. The efficiency of absorption by the gastrointestinal tract is highly influenced by the physical form of this compound and on the method used to suspend or dissolve it for administration. This observation is based on acute oral studies with rats (Schneider *et al.*, 1976). Ten rats dosed with 100 mg RDX/kg in a coarse, granular saline slurry had no convulsions. However, five rats receiving the

same dose as a suspension in one percent methyl cellulose all convulsed and died. Furthermore, all ten rats that received RDX at the lower dose of 50 mg/kg convulsed and two died. The acute LD50 of RDX is therefore dependent upon the physical form of RDX and the method used to prepare it for administration. Since no controls were reported for these observations, and only a small number of animals was tested, these conclusions are not necessarily valid.

There is no evidence of skin absorption of RDX in rats (Taylor, 1975). In rabbits, single doses of 1.0 ml RDX in different solvents (33% w/v RDX in DMSO; 5.4% w/v RDX in acetone and 7.5% w/v RDX in cyclohexanone) applied topically showed no significant alterations in blood red and white cell count, hematocrit, hemoglobin, urea nitrogen, creatinine, alkaline phosphates, serum glutamic-oxalacetic transaminase or electrolytes. Sporadic deaths occurred after repeated doses (McNamara *et al.*, 1974). Controls were performed in these studies, but there was no indication of when blood samples were drawn for analysis. In addition, only six animals were used for each test group. More animals need to be tested to properly state that there were no significant alterations in the blood parameters of experimental animals.

When dogs received either acute, subacute or massive subacute percutaneous doses of RDX in one of three solvents, there were no consistent alterations in blood pressure, heart rate, respiration, EKG or EEG (McNamara *et al.*, 1974). Acute doses were 65.7 mg/kg (7.5% RDX in cyclohexanone), 97.3 mg/kg (5.4% RDX in acetone), and 289.0 mg/kg (33% RDX in DMSO). The subacute study used the same doses as the acute study, but dosing was carried out 5 days/week for four weeks. For the massive subacute study, animals received 480 mg/kg of 33% RDX in DMSO for three consecutive days. Although the appropriate controls were performed, only certain results were documented in tables. For more accuracy, more animals should have been tested and all the results tabulated.

b. Transport

One major study has been conducted in order to determine the distribution of RDX in the mammalian body (Schneider *et al.*, 1976). Male and female randomly bred Sprague-Dawley derived rats and female miniature swine were utilized as test subjects in this study. Ten rats were dosed with 500 mg of RDX/kg by the intraperitoneal route and two rats with 50 mg RDX/kg. In addition, groups of 10 rats were given 100 mg RDX/kg by oral gavage. Groups of rats were euthanized at specific times up to 24 hours after dosing, and tissue samples were taken for RDX analysis at death or upon sacrifice of the animals. The results of these analyses are presented in Table I-3 as mean value, plus and minus standard error.

Examination of the table shows that RDX is not preferentially distributed to any one tissue. However, in rats, the kidneys did contain a consistently larger amount of RDX than the other tissues. Of the tissues examined, the rat liver varied the most widely in RDX content as a function of time after dosing.

Table I-3. Concentration of RDX in Various Rat Organs as a Function of Time After Dosing (Schneider *et al.*, 1976).

Animal	Rat	Rat	Rat	Rat	Rat	Rat	Rat	Rat	Rat	Swine
No. of Animals	10	2	10	10	10	10	10	10	10	10
Time	Death or 3.5 or 6.5 hr	2 hr	2 hr	4 hr	6 hr	8 hr	12 hr	18 hr	24 hr	24 hr
Dosage	500 mg/kg i.p.	50 mg/kg i.p.	100 mg/kg oral gavage	100 mg/kg oral gavage	100 mg/kg oral gavage	100 mg/kg oral gavage	100 mg/kg oral gavage	100 mg/kg oral gavage	100 mg/kg oral gavage	100 mg/kg oral gavage
Brain µg/g	29.5±2.7	3.7±0.6	10.36±1.24	7.71±0.98	7.51±0.57	5.57 0.67	11.28±1.6	6.30±0.20	8.91±1.07	7.0±1.0
Heart µg/g	25.0±2.3	2.8±0.1	7.97±1.11	6.49±0.96	7.13±0.71	3.82±0.52	11.08±1.82	5.58±2.24	7.89±0.83	5.3±1.4
Liver µg/g	41.7±5.8	5.8±3.8	4.34±0.90	2.16±0.56	0.51±0.34	0.15±0.15	8.51±2.24	0.48±0.20	2.56±1.15	4.4±1.7
Kidney µg/g	56.8±5.9	9.4±0.4	12.86±1.40	12.30±1.82	13.58±1.37	10.90±0.86	22.02±2.06	12.12±0.83	16.85±0.8	Cortex 9.1±4.3 Medulla 5.6 1.8
Plasma µg/g	13.8±2.6	1.1±0.04	1.50±0.26	2.09±0.09	1.78±0.15	2.36±0.22	2.28±0.16	2.03±0.10	3.04±0.48	4.7±2.1

Since these results were presented with their standard errors, they are statistically valid, even though a relatively small number of animals were tested.

c. Metabolism

The ability of rats to metabolize RDX was studied by Schneider *et al.* (1976). Radio-labeled RDX was administered by oral gavage at doses of 50 mg/kg. Tissue examination of sacrificed animals at various intervals up to 96 hours after dosing revealed that recovery of radioactivity from the various tissues diminished with time. Recovery was less than 50 percent at 96 hours. The liver RDX content varied widely during this time. Furthermore, both the liver and urine contained greater amounts of ^{14}C than could be accounted for by RDX content of the tissue alone. Therefore, the liver and urine contain significant quantities of a metabolite(s) of RDX and it appears likely that metabolism of RDX occurs in the liver.

In a second study (Schneider *et al.*, 1976), 30 rats were dosed with 50 mg/kg by oral gavage. Ten of the rats were used for urine collection, 10 for $^{14}CO_2$ collection and 10 for whole-body homogenates at 96 hours after dosing. At 4 days, radioactivity recovery was ~ 90% with 3% in the feces, 10% in the carcass, 34% in the urine and 43% as $^{14}CO_2$. Therefore, a relatively efficient mechanism must exist for metabolism of RDX *in vivo* since only 4% of the administered dose was recovered unmetabolized in either the urine or feces. One mechanism of metabolism must include ring cleavage, as evidenced by the large portion of labeled CO_2 produced. These results are in direct conflict with earlier findings of Sunderman *et al.* (1944). They stated that the entire dose of RDX fed to two rats was excreted unmetabolized in the feces. The differences in the results of these two studies can most probably be attributed to analytical techniques, but it must also be remembered that results of a study using only two animals should carry less weight than a study utilizing 30 animals. Schneider *et al.* (1976) utilized radio-labeled RDX and gas chromatography for quantitative analysis. Sunderman hydrolyzed the RDX to nitrite and determined the levels colorimetrically. This method is subject to a variety of interferences which tend to increase apparent RDX levels. TAX would also be expected to undergo metabolism with initial cleavage of the amide group followed by ring cleavage.

d. Elimination

RDX elimination was followed in 10 rats dosed with 50 mg RDX/kg by oral gavage (Schneider *et al.*, 1976). The plasma, urine and feces were monitored daily for 14 days. The concentration of RDX in the plasma and urine reached peak levels one day after dosing. The urine levels of RDX at this time were approximately twice that of the plasma. Both plasma and urinary RDX levels rapidly declined on the second through the fourth day after dosing; they then maintained a low steady state of <1 mg/ml from the fourth through the thirteenth day. In general, urinary RDX levels remained higher than plasma levels. Total urinary excretion during the first six days was 2.37 ± 0.15 percent of the administered dose; the estimated fecal excretion of RDX during that time was only 0.69 ± 0.05 percent.

Ten female miniature swine were dosed with 100 mg finely powdered RDX/kg by oral gavage (Schneider *et al.*, 1976). Plasma, urine and feces RDX levels at 24 hours were:

Plasma:	4.7 ± 2.1 µg/ml
Urine:	3.6 ± 1.1 µg/ml
Feces:	2.5 ± 0.6 µg/g

In contrast to the rat, urine levels were less than or approximately equal to the plasma levels. For comparison of the swine data to the data for rats, levels of RDX in the swine should have been monitored for more than 24 hours. In addition, amounts excreted in the swine should have also been presented as percent of administered dose excreted. Presentation of standard errors is a valid statistical analysis for a small number of animals.

e. Pharmacology

The main outward symptoms of RDX intoxication indicate that this compound causes injury to the central nervous system. Several studies have been conducted in order to determine the pharmacological actions of RDX and provide a warning sign for RDX intoxication. In a study by von Oettingen *et al.* (1949), seven dogs were given 50 mg/day, six days a week for six weeks. Excitability and irritability were observed after the first dose. Within the first week, the animals had convulsions which were characterized by hyperexcitability, increased activity, chronic movement and salivation. As dosing continued, tonic convulsions and collapse were observed. One animal died, and all animals lost weight.

The absence of overt cytotoxicity indicates that the action of RDX may be biochemical. This hypothesis was investigated in a study in which Sprague-Dawley rats were dosed daily for 12 weeks by intraperitoneal injections of RDX in either oil or carboxymethyl cellulose. Daily dosages ranged from 0.3 to 6.5 mg/kg, administered as 1 ml solution/kg/day (Maryland University, Baltimore School of Pharmacy, 1975). Body weights, blood levels of RDX, and indirect neurological symptoms were monitored. Upon sacrifice, brain oxygen uptake and levels of cholinesterase and monoamine oxidase were determined, since changes in the activities of these enzymes are established indices of CNS toxicity. Brain steady-state and turnover rates for the neurotransmitters norepinephrine, dopamine, and 5-hydroxytryptamine were also measured. Results of the study showed that chronic administration of RDX produced transient changes in biochemical parameters of the CNS at doses which produced no obvious signs of toxicity. Time and dose related changes were evident in brain monoamine oxidase and cholinesterase levels and oxygen uptake. The variations of these and other parameters at different times during the study for the highest dose rate (6.5 mg/kg/day) are presented in Table I-4. Similar changes were observed for lower dose rates, although variation was not as great as for higher rate. From Table I-4, it can be seen that the concentration of RDX in the blood correlated with the activities of the two enzymes. However, there was no correlation of either blood levels of RDX or enzyme activity with changes in oxygen uptake or levels of neurotransmitters.

Table I-4. Biochemical Parameters Observed in Rats Administered 6.5 mg RDX/kg/day for 12 weeks (Data from Maryland University Baltimore School of Pharmacy, 1975)

Parameters Measured	Time in Study			
	24 Hours	2 Weeks	6 Weeks	12 Weeks
Mean RDX Conc. in Whole Blood, mg/ml	0.111	0.098	0.134	0.222
Monoamine Oxidase Activity (% of Control)	85	70	180	200
Cholinesterase Activity (% of Control	85	70	140	180
O_2 Uptake (% of Control)	110	95	175	100
Norepinepherine (% of Control)	95	135	90	100
Dopamine (% of Control)	110	65	90	75
5-Hydroxy-tryptamine (% of Control)	80	110	80	100

Although this study was designed as a Latin Square for each biochemical parameter, and results were presented as mean values with ranges, the results were confusing. Several statements in the text made about trends of parameters disagreed totally with data and graphs presented. Discrepancies of this nature do not add to the credibility of the study.

Beagle dogs fed 0.1, 1.0 and 10 mg/RDX/kg/day for 90 days developed no signs of toxicity other than minor vomiting and nausea, to which a tolerance developed (Litton Bionetics, Inc., 1974a). Each dog was observed on a daily basis for signs of intoxication. Hematological, biochemical and urinalysis procedures were performed twice before dosing and during the 4th, 8th and 12th weeks of the study. The hematological procedures included measurements of hematocrit and hemoglobin levels, erythrocyte, total and differential leukocyte, reticulocyte and Heinz body counts and red blood cell fragility. Biochemical studies included fasting blood sugar; blood urea nitrogen; methemoglobin; total serum protein; total serum bilirubin; serum sodium, potassium glutamic-pyruvic transaminase, alkaline phosphatase, and glutamic-oxaloacetic transaminase; and sulfobromophthalein liver function test. The urine was tested for pH, specific gravity, glucose, ketones, total protein, bilirubin, glutamic-oxaloacetic transaminase and examined microscopically. No abnormalities were observed in these parameters over those which occurred in the controls. After the 90-day period, the dogs were sacrificed and the individual organs removed, weighed and subjected to histopathological examination. No effects which could be attributed to RDX intoxication were observed.

In a second study by Litton Bionetics, Incorporated (1974b), rhesus monkeys were given 0.1, 1.0 and 10.0 mg RDX/kg/day for 90 days by oral gavage. The animals were observed daily for signs of intoxication. Five out of six monkeys on the highest dose of RDX showed instances of CNS disturbance, usually involving tonic convulsions. One of these monkeys had to be euthanized; the others recovered and survived the study. Other than frequent instances of vomiting, especially in the high dose group, no other signs of intoxication were observed. Hematological, biochemical and urinalysis procedures were performed three times prior to dosing, during the 5th and 9th weeks of the study, and just after administration was stopped. Parameters measured were similar to those mentioned above in the beagle study. Laboratory testing revealed only scattered changes of no toxicological significance.

2. Effects of Human Exposure

No specific instances of occupational exposure to TAX have been reported in the literature. Only a few instances of occupational exposure to RDX have occurred, despite the fact that this compound has been manufactured for at least a quarter of a century. The low incidence of such exposure is probably the result of adequate safeguards being taken to prevent workers from coming in contact with RDX.

Barsotti and Crotti (1949) reported 17 cases of exposure that occurred among Italian workers engaged in the handling of RDX at various stages of its manufacture. Of these 17 cases, 10 suffered loss of consciousness without convulsions, two had vertigo and 1 had vomiting and mental confusion. These effects either occurred without any warning symptoms or were preceded by several days of insomnia, restlessness, irritability or anxiety. A transient arterial hypertension was present in all of these cases. Recovery in all these cases was complete and without event.

Vogel (1951) described similar cases that occurred in German workers who had been handling finely pulverized RDX powder. It was pointed out that the finer the dust, the earlier the adverse reactions appeared. Further, it was noted that if a worked vomited at the onset of illness, the symptoms were mild. However, if vomiting was absent, sudden attacks of unconsciousness and convulsions occurred. These were followed by exhaustion and decreased pulse and blood pressure. Unconsciousness, which lasted from minutes to an hour, was followed by vertigo and malaise for several days.

A third report includes five workers who sustained exposure to RDX at a U.S. explosives plant (Kaplan *et al.*, 1965). Four of the five workers were engaged in barrel-emptying, drying of RDX and dumping RDX in cans. The fifth worker was involved in the screening and blending of RDX. When the operation began at this plant, no mechanical ventilation was provided. Subsequently, overhead canopy hoods and an exhaust-ventilated hood were installed. Exposure among these five workers ranged from one day to six months. Their ages ranged from 18 to 26 years. Symptomology of RDX intoxication was similar to that reported above. Either at work, or several hours after returning home, the workers would have a sudden convulsion or become unconscious without a convulsion. Warning signs were few or non-existent. Some of the patients would experience only short periods of headache, vertigo, nausea, and emesis. Following these symptoms, there was a period of unconsciousness that lasted from several minutes to 24 hours. Upon regaining consciousness, the vomiting and weakness ensued. Examination of the workers showed no abnormal physical findings except those relating to the CNS; and no changes were found in the complete blood chemistry test or urine analysis. Treatment was supportive and recovery without event. Re-exposure of two of the men to RDX resulted in recurrence of the illness.

3. Effects on Experimental Animals

a. Acute Toxicity

The acute toxicity of RDX has been determined on a variety of experimental animals by several different methods of administration. Results of these studies are compiled in Table I-5. In view of the other data presented, the oral LD50 of 500 mg/kg in mice appears to be too high.

Care should be exercised in comparing LD50 found in various studies since this dose has been shown to depend upon the physical form of the chemical when administered (Schneider, *et al.* 1976).

Table I-5. Acute Toxicity of RDX to Mammals

Animal	Method of Administration	Dose	Response	Reference
Rat (*Rattis norvegians*)	Oral	152.6 mg/kg	LD50	Kaczorowski and Syrowatka, 1960
Rat (Sprague-Dawley derived)	Oral in a coarse, granular saline slurry	300 mg/kg	LD50	Schneider *et al.*, 1976
"	Oral in a fine saline slurry	100 mg/kg	LD50	"
"	Oral in DMSO	100 mg/kg	LD50	"
Rat (non-fasting)	Oral	200 mg/kg	LD50	Sunderman *et al.*, 1944
Mice	Oral	500 mg/kg	LD50	Sklyanskaya and Pozhariskii, 1944
Mice	i.v. in DMSO	18.7 mg/kg	LD50	McNamara *et al.*, 1974
Guinea Pigs	i.v. in DMSO	25.1 mg/kg	LD50	"

b. Subacute Toxicity

A study to determine the subacute toxic effects of RDX on the brains of male rats was conducted at the University of Maryland School of Pharmacy (1975). Preliminary results showed that intraperitoneal injection of 0.3, 2.5 and 6.5 mg RDX/kg caused a decrease in body weight at 2 weeks, but that the weight returned to normal levels by six weeks, and continued at that level through the 12th week. Thus, rats receiving continuous doses of RDX appeared to develop a tolerance to it during the first three to four weeks.

Blood levels of RDX, 24 hours after dosing, were related to dose administered and duration of exposure. Alterations in brain enzyme activities were directly associated with increased blood levels of RDX (See Table I-4). However, changes in neurotransmitter levels were not correlated with variations in enzyme levels. Thus, in addition to producing changes in oxygen uptake and increases in monoamine oxidase and cholinesterase, subacute exposure to RDX also increased blood levels of this compound. These changes were dose related. Rats receiving the lowest dose (0.3 mg/kg/day) showed similar trends of various biochemical parameters as the highest dose (6.5 mg/kg/day; Table I-4), but the value of these parameters was proportionately less for the lower dose.

It is unfortunate that the only data available on the neurological effects of RDX is contained in this study. Discrepancies between data, tables, graphs and text detract from the credibility of the results.

Litton Bionetics, Incorporated (1974a) conducted a study of the subacute toxicity of RDX in purebred male and female dogs. These animals were fed daily doses of 0.1, 1.0 or 10.0 mg/kg for 90 days. No signs of toxicity occurred other than scattered episodes of vomiting; no changes in body weight beyond control fluctuations were found. A variety of hematological, biochemical, and urinalysis tests were carried out in these animals at various times during the study. Details of these tests are given in Section D.1.e.

In addition to the laboratory procedures, all animals were necropsied. The following organs were removed and weighed: heart, kidneys, liver, thyroids, adrenals, spleen and testes with epididymis. In addition, selected tissues from the control and high dose (10 mg/kg) group were examined for histopathological changes; these tissues include: brain, thyroid, lung, heart, liver, spleen, kidney, adrenal, stomach, small intestine, bone marrow, and any lesions found. Results of laboratory diagnostic procedures and histopathologic examination revealed no remarkable changes.

Opthalmoscopic examination of the dogs revealed no changes attributable to RDX. In the high dose group (10 mg/kg), there was some occurrence of increased granularity and mild hyper-reflectivity of the fundus, but this was not considered to be an important indication of toxicity.

The subacute toxicity of RDX was evaluated in rhesus monkeys using oral administration of 0.1, 1.0 or 10.0 mg RDX/kg/day, seven days a week for 13 weeks (Litton Bionetics, Incorporated, 1974b). Daily observations were

made for signs of toxic effects, and laboratory procedures included hematology, clinical biochemistry, and urinalysis. See Section D.1.e for a detailed description of these procedures.

Frequent episodes of vomiting were present among animals in the high dose (10 mg/kg) group. In addition, five out of six animals in this group displayed CNS disturbance, usually in the form of tonic convulsions. Other than these disturbances, no other changes of toxicological significance were noted, and opthalmoscopic examination revealed no effects on the eyes due to administration of RDX.

In the bone marrow of monkeys given the high level of RDX, histopathologic examination revealed some increase in the number of degenerate or necrotic megakaryocytes. Increased amounts of iron-positive material were also found in the liver cord cytoplasm. The toxicological significance of these findings is uncertain at the present time (Litton Bionetics, Incorporated, 1974b).

In studies of subacute toxicity on dogs and rats, von Oettingen *et al.* (1949) found that the only consistent change produced as a result of ingesting RDX was weight loss, convulsions and hyperirritability. Repeated daily administration to rats of 25, 50 or 100 mg RDX/kg/day for three months produced mortality of 40 to 86.6%; feeding of 15 mg/kg did not cause mortality. Repeated exposure to 25 and 50 mg/kg did not cause changes in the blood counts or histologies of rats; no hematology or histologic studies were made of the 100 mg/kg group. In dogs fed 50 mg/kg six days a week for six weeks, weight loss, hyperirritability and convulsions were produced. No changes in blood chemistry, blood count or histopathology were observed.

Although von Oettingen used a wide range of doses for his feeding studies, and performed relatively complete hematologic and histopathologic profiles on his subjects, it is unfortunate that he chose not to do so in the highest dose group. Observations from this group would have made the study more complete.

The gross toxicological effects of RDX are expected to be similar to TAX, due to the similarity of their structures. (See introduction to Section D.) It is therefore expected that subacute exposure to TAX may result in vomiting, CNS disturbance and weight loss. TAX is expected to have a more increased biological activity than RDX, so the severity of these effects may be increased.

c. Chronic Toxicity

A detailed study of the effects of feeding RDX to 800 Charles River Sprague-Dawley rats at the levels of 1.0, 3.1 and 10.0 mg/kg for 2 years provided no important evidence of toxicity (Hart, 1976). Clinical studies were performed on 10 female and 10 male animals from each control and each test group. Hematology (at 13, 26, 52, and 104 weeks) included hematocrit, hemoglobin, erythrocyte count, total leukocyte count, differential leukocyte count, and reticulocyte count. Blood chemistry (at 52 and 104 weeks) included

fasting blood sugar; blood urea nitrogen; total serum protein and bilirubin; serum sodium, potassium, chloride, glutamic-pyruvic transaminase, alkaline phosphatase, glutamic-oxalacetic transaminase and methemoglobin. Urinalyses, using pooled samples at 13, 26, 52 and 104 weeks, included pH, specific gravity glucose, ketones, bilirubin, microscopic examination of sediment and urine glutamic-oxalacetic transaminase.

At 52 weeks, 10 female and 10 male animals from the control and each of the three test groups were "sacrificed" and necropsied. All organs were preserved in buffered 10% formalin, and weights were recorded for liver, kidneys, thyroids, and adrenals. At 104 weeks, all surviving rats were "sacrificed" and necropsied.

Histopathological examination of tissue from all animals in the control and high level group included pituitary, thyroid, heart, liver, spleen, kidney, adrenal, stomach, pancreas, small and large intestine, mesenteric lymph node, urinary bladder, testes, ovary, bone marrow, and any unusual lesions. For the intermediate and low level groups, the liver, kidney, thyroid and adrenal tissues were examined microscopically. Pathological examinations were made of the organs of those animals that died during the course of the study.

Results of this study showed no significant toxicity with regard to mortality, body weight, food consumption, hematocytology, blood chemistry, urinalysis, postmortem organ weights, gross necropsy findings, or histopathology.

This massive study was extremely well planned, executed, and documented. In addition, a large enough number of animals was tested to ensure statistical accuracy. Other such studies would do well to follow a similar format.

d. Teratogenicity and Mutagenicity

No studies on the teratogenic effects of TAX or RDX were found in the literature.

The Ames bacterial mutagenicity test was performed on Holston AAP wastewater effluents (Stilwell *et al.*, 1977). A set of five *Salmonella typhiumurium strains,* TA-1535, TA-1537, TA-1538, TA-98 and TA-100, were used. The histidine deficient variant strains are used to detect frame shift reverse mutations (TA-1537, 1538, and 98) or base pair substitutions (TA-1535, and 100). A dose response curve ranging from 5 ml to 100 ml of test water was used for each strain. An index of relative mutagenicity which has test reversions/ control reversions was used. The value of 1.0 indicated no mutagenic activity with 10 clearly indicating mutagenic activity. The highest wastewater index was 2.07 and was attributed to chance variation. Therefore, Stilwell *et al.* (1977) found no mutagenic properties in Holston AAP effluents.

e. Carcinogenicity

No evidence of any carcinogenic potential was uncovered in any of the toxicological studies with RDX.

f. Behavior-Symptomology

RDX and presumably TAX intoxication in humans results in a variety of symptoms all of which are associated with central nervous system injury. These symptoms include confusion, hyperirritability, convulsions, unconsciousness, prolonged mental confusion and amnesia. Convulsions usually occur several hours after the intoxication. Experimental animals receiving acute or chronic doses of RDX exhibit the same general symptoms. In rats and dogs, convulsions tend to occur soon after dosing. However, miniature swine behave more like humans in that convulsions do not occur until many hours after dosage (Schneider *et al.*, 1976). Although no correlations were found comparing RDX to TAX, it is assumed that TAX will also cause central nervous system injury, due to the similarity.

E. Environmental Effects

1. Entry into the Environment

The only source of entry of TAX into the environment in the United States is in the wastewaters from the RDX/HMX production at Holston AAP. These wastewaters currently flow either directly or individually into the Holston River. TAX is discharged mainly from the dewatering and incorporation steps. Average concentration of TAX in the effleunt streams at N-5 and N-6 are 4.8 and 2.6 mg/l (see Section C.2. for details). TAX concentrations in these streams range from 60 to 90% of the RDX concentration. Based on the 1977 sampling and analysis, up to 1.3 to 1.9 million lb/year of TAX could be discharged from Holston AAP under full mobilization operations.

2. Behavior in Soil and Water

a. Transport and Accumulation

The movement of TAX in the environment has not been reported. Until a data base is developed, predicting the fate of TAX must be done from available information on RDX.

Sikka *et al.* (1978) reported the solubility of RDX in water at 20°C to be 42.3 ppm. TAX, with the substitution of an acetyl group for a nitro group, should have a slightly higher solubility.

Sikka *et al.* (1978) measured the adsorption of RDX on three sediment types. As shown in Table I-6, RDX has low partition coefficients for adsorption onto sediments. Even with low adsorption, levels of RDX in the sediments reached a steady-state concentration, between 30-40 ppm in the organic and clay sediments. Sikka *et al.* (1978) used a 1:100 adsorbant: solution ratio. However, the EPA (Federal Register, 1979b) recommends a 1:5 ratio. Increasing the amount of sediment could increase the adsorption values for RDX onto the sediment.

Sullivan *et al.* (1977) found the average level of RDX in the combined effluents of Holston AAP to be 3.95 ppm. The highest RDX levels found in the Holston river water was 70 ppb. They also analyzed the sediment in the Holston River for RDX, but did not find the compound over the detection limit of 0.2 ppm. Sikka *et al.* (1978) in laboratory studies on sediments, found that 30-40 ppm was a steady-state concentration. RDX and TAX concentrations in the sediments of the Holston River should be in the ppm range.

The absence of RDX, and probably TAX in the sediment of the Holston River near Holston AAP is probably due to scouring of the sediment (Sullivan *et al.*, 1977). This scouring occurs because of the wide fluctuation of water levels in the river due to Ft. Patrick Henry Dam. As shown in Figure I-2, the water levels in the Holston River vary by 10 fold daily. Thus, any chemicals which accumulate in the sediment near Holston AAP are rapidly washed down river when the dam gates are open.

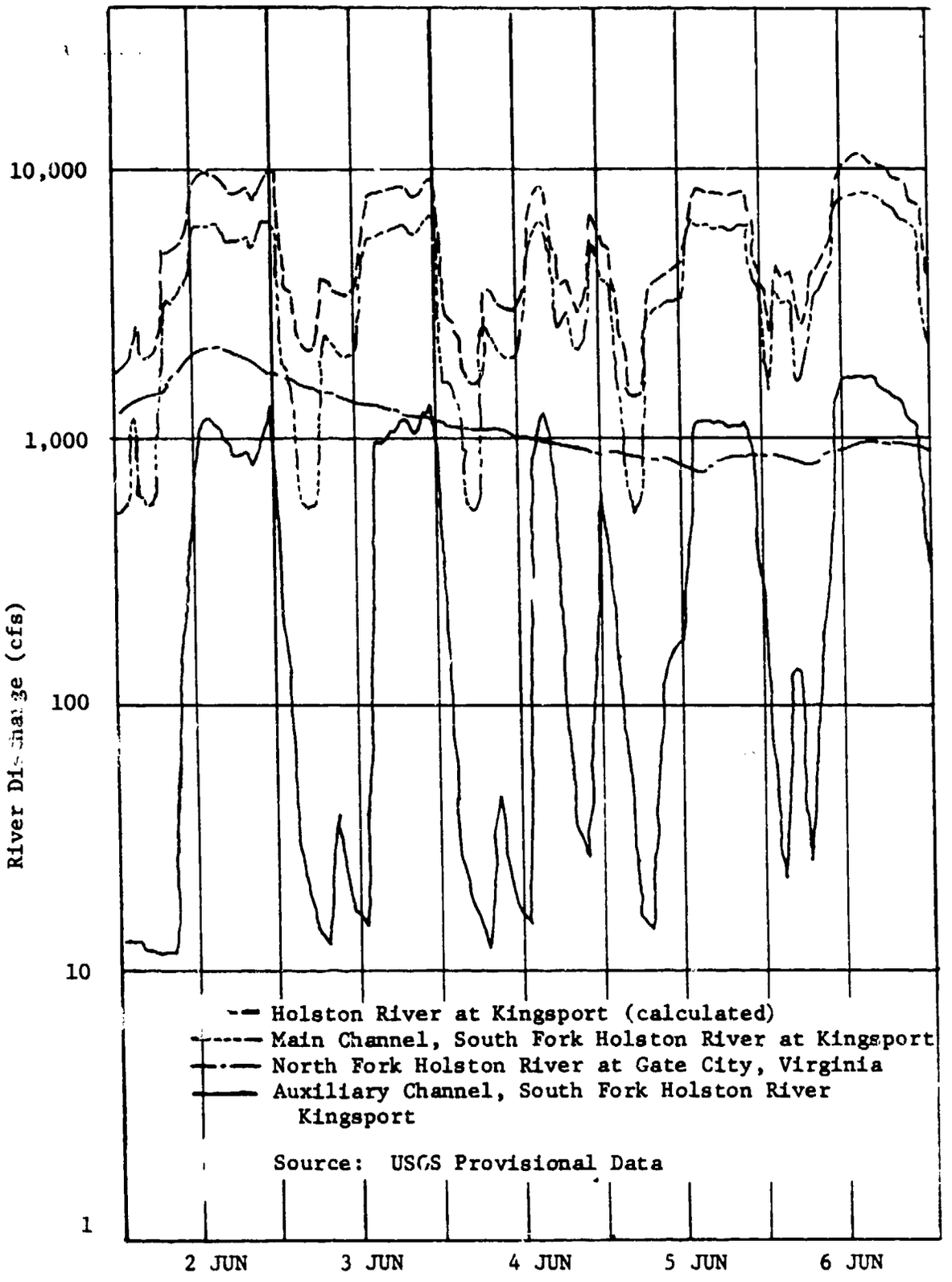


Figure I-2. Streamflow Data for the Holston River and Tributaries at Kingsport. June, 1975 Sampling Trip. (Sullivan *et al.*, 1977)

Table I-6. Adsorption of RDX by Sediments
(Sikka *et al.*, 1978)

Sediment	Partition Coefficient (K_p)
Organic Muck	4.15
Clay Loam	3.06
Sandy Loam	0.80

b. Degradation

Sikka *et al.* (1978) studied the photolysis of RDX exposed to UV light around 230 nm. The tests were apparently conducted in distilled or tap water. The results indicated, Table I-7, that RDX was substantially degraded within five hours. Also nitrite and nitrate concentrations increased during the irradiation. In sunlight, the photolysis of RDX procedes at a much slower rate. The data, in Table I-8, indicate that RDX is removed from the solution at a rate between .11-.27 ppm/hr. However, Spanggord *et al.* (1979) studied the photolysis of RDX and TNT together and found that rate of reduction of RDX was reduced approximately five times to .03 ppm/hr.

However, the removal of RDX or TAX in the environment through photolysis is doubtful. Wetzel (1975) stated that while distilled water absorbs small amounts of UV light, low concentrations of dissolved organic compounds absorb large amounts of the UV light. The data in Table I-9 indicate an almost total adsorbtion of UV, blue and green wavelengths in lakes with high dissolved organic levels. Sullivan *et al.* (1977) reported total organic carbon levels in the Holston River ranged from 3-330 mg of carbon/l. Total dissolved solids ranged from 62-576 mg/l in the river. The RDX or TAX present in the water would have to compete with other dissolved organic compounds for the UV light. In addition, within a water depth of 1 meter essentially all of the UV light would probably be absorbed.

The hydrolysis of RDX has been found to be a slow process. Sikka *et al.* (1978) found no hydrolysis of RDX under acidic conditions, over a 12-day period. However, they found 27% of the RDX was hydrolyzed under basic conditions (Table I-10). The hydrolysis of TAX, because of the acetyl group, should be less complete than RDX or HMX.

The loss of RDX due to volatilization in the environment was estimated to not be a major factor in the fate of this compound (Spanggord *et al.*, 1979). Because TAX and RDX are solids at 20°C and have high boiling points, it is likely that the volatilization of TAX is also low.

c. Background Concentrations

Background concentrations of TAX in natural areas are non-existent.

3. Biodegradation and Bioconcentration

a. Biodegradation

No data is available in the literature on the degradation of TAX. However, the replacement of a nitro group by an acetyl group should render the molecule more susceptible to degradation by microorganisms. Osmon and Klausmeier (1973) tried to isolate organisms capable of using RDX as the sole source of carbon. They found organisms capable of growing on a complex media saturated with RDX, but no breakdown of RDX was observed.

Table I-7. Photolysis of RDX
(Sikka *et al.*, 1978)

Minutes of Irradiation	RDX x 10^5M	RDX ppm	(NO_2^-) x 10^5M	(NO_3^-) x 10^5M	(NO_2^-)/ (RDX)
0	12.0	26.6	0.093	2.1	
90	11.0	24.4	2.3	3.6	0.45
135	10.0	22.2	3.5	5.8	0.59
195	8.3	18.4	4.3	5.8	0.88
225	7.2	16.0	4.6	5.5	1.1
275	6.6	14.7	5.3	5.4	1.0

Table I-8. Photolysis of RDX by Natural Sunlight

Hours of Irradiation	RDX levels (ppm)	RDX Removal/ hour	Reference
0	3.82	-	Sikka *et al.* (1978)
0	3.89	-	"
2.3	3.22	.27	"
4.3	3.01	.20	"
6.8	2.70	.17	"
0	44	-	Spanggord *et al.* (1979)
72	36	.11	"
120	27	.14	"
168	21	.14	"

Table I-9. Percentile Absorption of Light of Different Wavelengths by One Meter of Lake Water, Settled of Particulate Matter, of Several Wisconsin Lakes of Progressively Greater Concentrations of Organic Color (Wetzel, 1975)

Wavelength (nm)	Distilled Water	Crystal Lake	Lake Mendota	Alelaide Lake	Mary Lake	Helmet Lake
800	88.9	89.9	90.5	92.4	91.7	93.2
780	90.2	91.3	91.9	93.5	93.0	94.5
760	91.4	93.5	92.6	94.5	94.8	96.0
740	88.5	89.3	91.5	92.7	93.0	96.2
720	64.5	67.6	71.0	78.0	78.0	86.9
700	45.0	50.4	49.7	66.3	70.7	82.5
685	38.0	45.2	42.2	65.7	71.7	86.6
668	33.0	40.3	36.8	65.0	72.3	88.0
648	28.0	37.0	31.9	64.5	75.2	91.2
630	25.0	34.4	28.9	65.8	77.8	94.0
612.5	22.4	32.1	26.3	66.8	80.3	96.0
597	17.8	27.5	22.5	67.0	83.2	97.6
584	9.8	22.0	17.6	67.1	85.7	98.2
568.5	6.0	19.3	14.0	67.6	88.5	98.6
546	4.0	19.2	13.5	70.9	91.6	99.3
525	3.0	19.8	14.1	74.5	94.8	*
504	1.1	20.7	15.2	81.0	97.4	*
473	1.5	21.7	21.7	88.6	99.4	*
448	1.7	23.8	27.8	92.2	*	*
435.9	1.7	24.4	31.0	95.2	*	*
407.8	2.1	28.1	44.3	99.0	*	*
365	3.6	40.0	80.0	*	*	*
Color Scale (Pt units)	0	0	6	28	101	264

*not measured

Table I-10. Hydrolysis of RDX at pH 9.07 at 31°C (Sikka *et al.*, 1978)

Days	% Hydrolysis[a]	$(NO_2^-) \times 10^6 M$	$(NO_3^-) \times 10^5 M$
0	0	3.3	-
3	4.1	4.0	5.2
5	6.4	4.7	4.5
7	9.2	5.6	-
11	16.7	9.2	-
14	22.5	10.5	2.7
18	25.4	11.9	-
21	27.0	13.0	3.8

[a] Based on RDX.

Kaplan (1979) observed disappearance of ^{14}C labelled RDX from anaerobically incubated cultures inoculated with activated sludge. 1,3,5-trinitroso-hexahydro-1,3,5-triazine was identified as the reduction product. No disappearance of ^{14}C-RDX was found in aerobically incubated cultures. Sikka *et al.* (1978) studied the microbial degradation of RDX. Water collected near an RDX waste outfall (lines 1-5) was spiked with RDX or with RDX and sediment. Results indicate an ∿20-day lag time before degradation initiated. Very little degradation was observed in samples without sediment. In samples containing sediment, nearly 80% of the RDX had disappeared within two weeks after degradation commenced (Sikka *et al.*, 1978).

It is expected that TAX will also be degraded by microorganisms in the environment. However, the extent to which the degradation will occur is not known.

b. Bioconcentration

The octanol-water partition coefficient (P) for TAX was calculated using the procedure outlined by Leo *et al.* (1971). Data for RDX were employed as the basis for calculating P for TAX. Octanol-water partition coefficients for RDX were obtained experimentally in Atlantic Research Corporation's laboratories and from bioconcentration factors (BCF) presented by Bentley *et al.* (1977). Experimental determination of P for RDX was made using the procedures outlined by EPA (Federal Register, 1979b). Solutions were maintained at 25°C. RDX analysis was carried out by high pressure liquid chromatography using a C_{18} μ-Bondapak column and a 30% methanol:70% water carrier phase. P for RDX was found to be 6.05. P for RDX was estimated from BCF presented by Bentley *et al.* (1977) using the following equation recommended by EPA (Federal Register, 1979a):

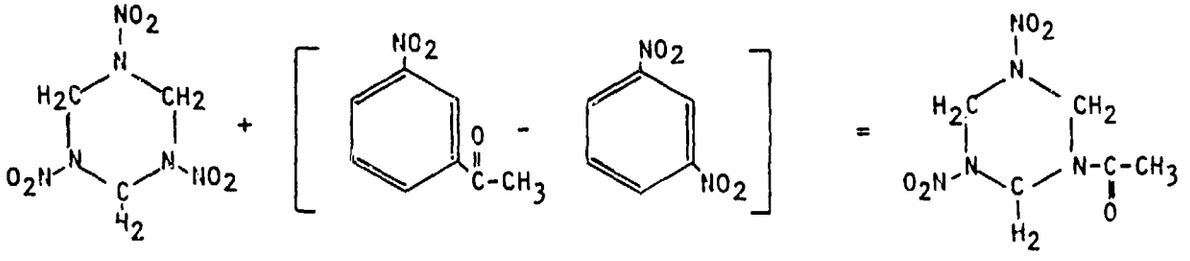
$$\log BCF = 0.76 \log P - 0.23$$

Using this equation and Bentley's experimental data, P for RDX was calculated to be between 5 and 41.

Calculations employed in determining P for TAX are presented in Table I-11. These calculations were performed using nitrobenzene and dinitrobenzene substitution data. Although this analogy is not strictly valid, the results should be reasonably close to the actual values. Estimates of the P and BCF for TAX are presented in Table I-12. Based on the data, TAX is not likely to bioconcentrate in aquatic organisms.

Table I-11. Calculation of log P for TAX

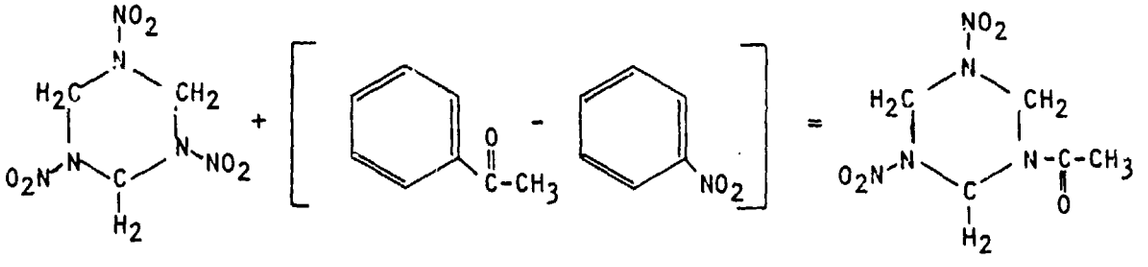
Method #1



RDX "TAX"

log P of RDX	+	log P of bracketed chemicals***	=	Estimated log P of TAX
.78*	+	[1.58 - 1.85]	=	.31
.70**	+	[1.58 - 1.85]	=	.43
1.61**	+	[1.58 - 1.85]	=	1.34

Method #2



RDX "TAX"

log P of RDX	+	log P of bracketed chemicals***	=	Estimated log P of TAX
.78*	+	[1.42 - 1.49]	=	.71
.70**	+	[1.42 - 1.49]	=	.63
1.61**	+	[1.42 - 1.49]	=	1.54

*Atlantic Research Corporation, 1979
**Bentley *et al.* (1977) calculated from BCF
***log P values from Fujita *et al.* (1964); technique, Leo *et al.* (1971).

Table I-12. Bioconcentration Factor (BCF) and Octanol-Water Partition Coefficients (P) for RDX and TAX

RDX P	RDX BCF	Estimated TAX Ranges P	BCF
41[1]	10.0[3]	21.9 - 35.0	6.1 - 8.7
5[1]	3.2[3]	2.7 - 4.3	1.3 - 1.8
6.05[2]	2.3[1]	3.2 - 5.1	1.4 - 2.1

[1]Calculated from log BCF = .76 log P - 0.23 (Federal Register, 1979a)

[2]Atlantic Research Corporation, 1979

[3]Bentley *et al.* (1977)

4. Effects on Animals

a. Mammals

There is no information on the effects of TAX on mammals in the environment. The available information of the effects of RDX on experimental animals was presented in Section D.3.

b. Birds

No information on the effects of RDX or TAX was found in the literature.

c. Fish

Stilwell *et al.* (1977) tested the toxicity of Holston AAP effluents to the fathead minnow (*Pimephales promelas*). RDX levels in the area A effluents ranged from 0.43 - 1.90 ppm. Estimated TAX levels range from 0.3 - 1.7 ppm. Although area A wastewater was acutely toxic, RDX levels did not correlate with the toxicity data.

No aquatic toxicity data on TAX are available in the literature. However, Bentley *et al.* (1977) conducted an aquatic toxicity study on RDX. The data generated on RDX should be applicable to TAX aquatic toxicity.

Toxicity tests were conducted on 4 species of fish to determine the 24 hr, 48 hr and 96 hr LD50's (Bentley *et al.*, 1977). The data are presented in Table I-13. Bentley *et al.* (1977) found the channel catfish to be the most sensitive with a 96-hour LC50 of 4.1 ppm. In a second experiment to determine the effects of water parameters on RDX toxicity, the water, pH, temperature and hardness were varied in the following manner:

pH	6.0	7.0	8.0
Temperature	15	20	25
Hardness	35	100	250

However, the toxicity of RDX to the bluegill (*Lepomis macrochirus*) was not significantly changed. A study was undertaken to determine the sensitivity of the eggs and post-hatch fry of the fathead minnow to RDX (Bentley *et al.*, 1977). As shown in Table I-14, the 7-day post-hatch stage was the most sensitive group with a 96-hour LC50 of 3.8 ppm.

The effects of continuous RDX exposure of the hatch, survival and length of fathead minnows are presented in Table I-15. At these concentrations, RDX did not significantly effect hatch or survival. Significant differences in mean lengths were reported. However, the statistical analysis of the data is questionable. Bentley *et al.* (1977) reported a significant

Table I-13. The LC50's of RDX to Fish in Static Toxicity Tests
(Bentley *et al.*, 1977)

Organism	Hours			Water Parameters		
	24	48	96	pH	Temperature °C	Hardness ppm as $CaCO_3$
Bluegill (*Lepomis macrochirus*)	14	8.5	6.0	7.1	20±1	35
Rainbow Trout (*Salmo gairdneri*)	9.4	7.0	6.4	7.1	14±1	35
Channel Catfish (*Ictalurus punctatus*)	7.5	6.0	4.1	7.1	20±1	35
Fathead Minnow (*Pimephales promelas*)	10	5.8	5.8	7.1	20±1	35

Table I-14. Acute Toxicity of RDX to Selected Life Stages of Fathead Minnows (*Pimephales promelas*) During Static Toxicity Tests (Bentley *et al.*, 1977)

Life Stage	LC50 (mg/l)			Water Parameters		
	24-hour	48-hour	96-hour	pH	Temperature °C	Hardness ppm as $CaCO_3$
Eggs	>100	>100	>100	7.1	20±1	35
1-hour post hatch	>100	>100	43	7.1	20±1	35
7-days post hatch	>32	18	3.8	7.1	20±1	35
30-days post hatch	18	16	16	7.1	20±1	35
60-days post hatch	11	11	11	7.1	20±1	35

Table I-15. Mean Percentage Hatch, Mean Percentage Survival and Total Length of Fathead Minnow (*Pimephales promelas*) Continuously Exposed to RDX.

Measured Concentration (mg/l)		Mean hatch (%)	30 days survival (%)	30 days total length (mm)
5.8	A	78	87.5	17 ± 3[a]
	B	66	85	17 ± 3
3.0	A	64	82.5	18 ± 3
	B	69	87.5	18 ± 3
1.2	A	71	92.5	19 ± 3
	B	78	92.5	19 ± 3
0.76	A	69	67.5	19 ± 4
	B	59	70	20 ± 3
0.26	A	79	100	19 ± 3
	B	56	80	19 ± 3
control	A	63	95	18 ± 3
	B	75	85	19 ± 2
solvent control	A	80	92.5	19 ± 3
	B	68	100	19 ± 3

[a] $F=11.8$, $F=0.05$, $(6,6)=4.28$; $d'=1.01$

difference (95%) in mean total length at 5.8 ppm. Sullivan *et al.* (1979), analyzing Bentley's results reported a significant difference (95%) in mean length at 3.0 ppm. Both groups statistically analyzed only means. The correct method would be to use a completely randomized block design (Anderson and McLean, 1974) in which three or four minnow lengths would be selected at random from each group. An ANOVA table would then be set-up with fish length versus RDX levels. This method would give a better estimate of fish length variation within each group and would be the correct statistical test. However, judging from the length standard deviations no significant differences would be determined for minnow length.

Continuous exposure chronic RDX tests were conducted on fathead minnows (Bentley *et al.*, 1977). Results of these tests are presented in Table I-16. Survival was reduced at RDX levels of 6.3 ppm. For 30 and 60 days no significant effects on the hatchability, survival or length of the minnows were found at RDX levels of 3.0 ppm or less. As shown in Table I-17 no significant differences in survival time or weight at RDX levels of 6.3 ppm or less were apparent in the second generation.

The data on the bioconcentration of RDX in fish are presented in Table I-18. RDX has a bioconcentration factor (BCF) in steady state ranging from 3.2 - 10.0 in fish tissue. The highest concentrations of RDX were found in the viscera. This observation correlates with RDX tissue concentrations observed in mammals. In mammals the liver and kidneys had the highest concentrations of RDX. As shown in Figure I-3, RDX levels in the water and fish muscle track each other with about 4 day delay in fish tissue concentration. During depuration, RDX tissue levels are reduced to 0 in approximately 14 days in water containing no RDX.

A maximum safe concentration of RDX in freshwater for protection of aquatic life was calculated from aquatic toxicity data by Sullivan *et al.* (1979). A 24-hour average safe value for RDX in freshwater should not exceed 0.30 ppm. TAX should have limits similar to this.

d. Amphibians

No information is available in the literature on the toxicity effects of RDX or TAX.

e. Invertebrates

No data are available on the toxicity of TAX to invertebrates. However, RDX should have a similar toxicity to invertebrates. Aquatic invertebrates are tolerant to RDX levels. The LC50's for RDX, Table I-19, are greater than 100 ppm. A chronic RDX toxicity study was conducted on *Daphnia magna*. The results of the study are presented in Table I-20. The survival of *Daphnia* was not effected at 20 ppm.

Table I-16. Effects on First Generation Fathead Minnows (*Pimephales promelas*) During Continuous Exposure to RDX (Bentley *et al.*, 1977).

Concentration (mg/l)		Hatch (%)	30 Days Survival (%)	30 Days Total Length (mm)	60 Days Survival (%)	60 Days Total Length (mm)
6.3	A	92	25*	22±3	15*	33±6
	B	93	28	21±2	15	33±2
3.0	A	97	98	20±4	98	30±5
	B	100	98	20±3	93	30±4
1.5	A	95	93	21±3	93	32±5
	B	97	95	21±3	95	31±5
0.78	A	92	90	21±3	90	31±4
	B	92	100	20±3	100	31±4
0.43	A	97	90	21±3	90	31±3
	B	95	98	21±3	98	30±4
solvent	A	95	93	21±3	95	32±5
control	B	92	93	20±3	93	31±3
control	A	97	95	21±3	95	30±3
	B	95	100	20±4	100	29±6

*Significant at the 95% level

Table I-17. Survival and Growth of Second Generation (F_1) Fathead Minnows After 30 Days Continuous Exposure to RDX. (Bentley *et al*, 1977).

Measured Concentration (mg/l)		Survival %	Total Length (mm) Mean ± S.D.	Wet Weight (g)
6.3	A	85	24 ± 2	0.115
	B	98	23 ± 3	0.092
3.0	A	88	23 + 3	0.126
	B	93	23 ± 2	0.109
1.5	A	83	23 ± 3	0.119
	B	73	24 ± 3	0.141
0.78	A	75	24 ± 3	0.133
	B	78	25 ± 3	0.141
0.43	A	90	24 ± 2	0.105
	B	90	24 ± 2	0.119
solvent	A	75	23 ± 2	0.110
control	B	95	24 ± 2	0.118
control	A	68	23 ± 2	0.112
	B	85	22 ± 2	0.091

Table I-18. Bioconcentration of RDX in Fish

Organism	Water Level (ppm)	Tissue Level (ppm)	Bioconcentration Factor	Reference
Bluegill				
(Lepomis macrochirus)				
Viscera	0.30	0.95	3.2	Liu and Bailey (1977)*
Muscle	0.30	0.59	2.0	" "
Viscera	0.013	0.13	10.0	Bentley *et al.* (1977)**
"	0.75	7.5	10.0	" "
Edible Tissue	0.01	0.048	4.8	" "
" "	0.74			
Channel Catfish				
(Ictulurus punctatus)				
Viscera	0.01	0.060	6.0	" "
"	0.75	4.1	5.5	" "
Edible Tissue	0.01	0.032	3.2	" "
Fathead Minnow				
(Pimephales promelus)				
Viscera	0.005	0.047	10.0	" "
"	0.77	7.7	10.0	" "
Edible Tissue	0.01	0.047	4.7	" "
"	0.75	3.1	4.2	" "

* Steady State not reached
**Levels at day 21 of test

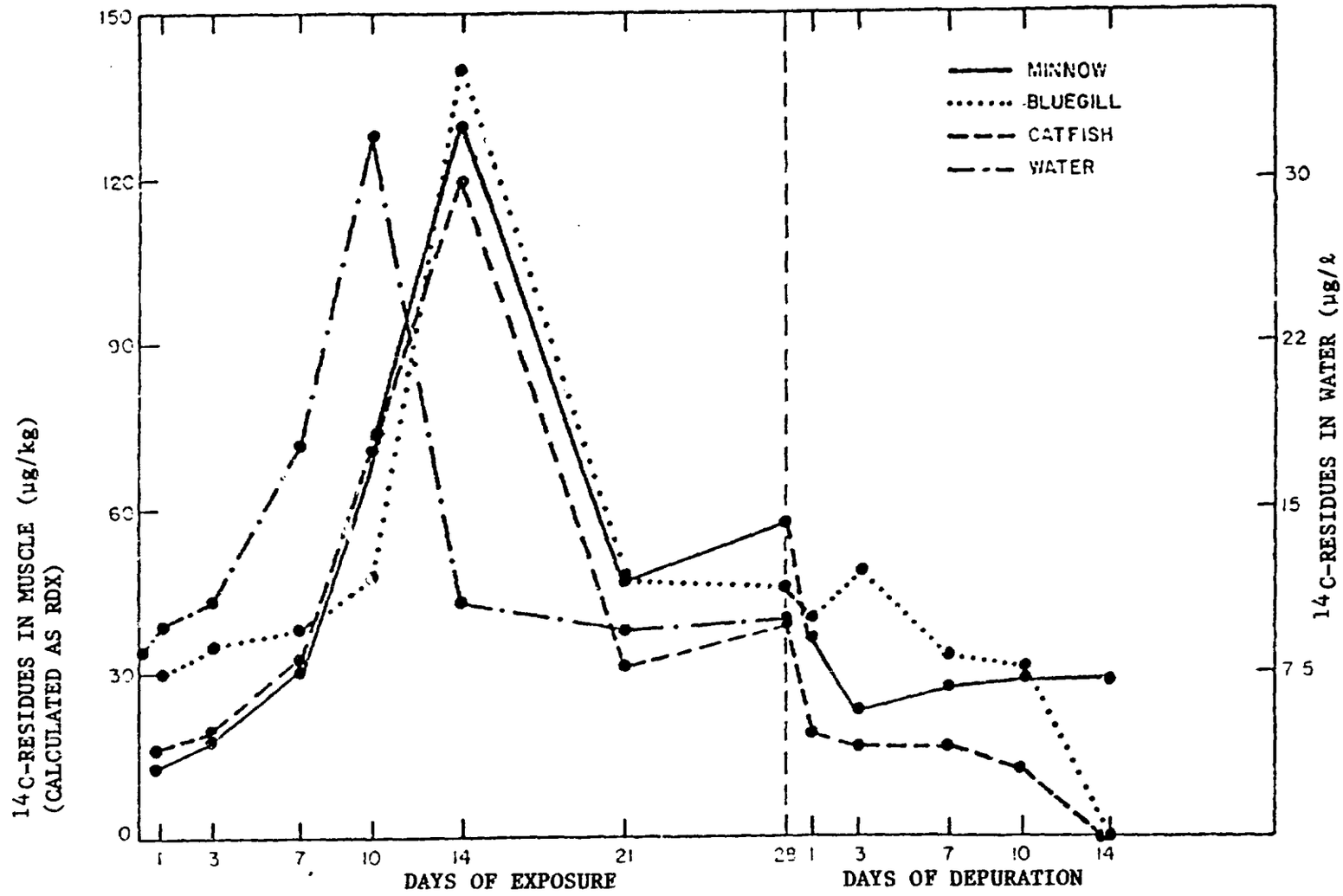


Figure I-3. Mean Measured ^{14}C-Residues, in the Water and in Fish Muscle During 28 Days Continuous Aqueous Exposure to a Nominal ^{14}C-RDX Concentration of 0.010 mg/1 and During 14 Days Depuration in Flowing, Uncontaminated Water (Bentley *et al.*, 1977).

Table I-19. Acute Toxicity of RDX to Aquatic Invertebrates in Static Toxicity Tests (Bentley *et al.*, 1977)

Species	Hours of Exposure 24	48	Water Parameters pH	Temperature (°C)	Hardness (ppm as $CaCO_3$)
Water flea (*Daphnia magna*)	>100	>100	7.1	20 1	35
Scud (*Gammarus fasciatus*)	>100	>100	"	"	"
Sowbug (*Asellus militaris*)	>100	>100	"	"	"
Midge larvae (*Chironomus tentans*)	>100	>100	"	"	"

Table I-20. Weekly Mean (Standard Deviation) Percent Survival of the Water Flea (*Daphnia magna*) Exposed to RDX (Bentley *et al.*, 1977)

Measured Concentration (mg/l)	Mean Percent Survival*					
	Generation 1			Generation 2		
	day/7	14	21	28	35	42
control	79(11)	78(12)	78(12)	82(13)	76(20)	76(20)
1.4	77(26)	78(26)	78(26)	86(14)	58(31)	58(28)
2.2	81(22)	81(22)	75(30)	92(9)	68(32)	62(35)
4.8	95(7)	76(35)	72(35)	91(12)	82(16)	74(22)
9.5	81(15)	80(15)	71(13)	92(15)	70(34)	69(34)
20	90(12)	86(11)	71(21)	95(10)	91(8)	90(8)

* Each survival value represents the mean of 4 replicate treatment vessels.

In a continuous exposure study, Bentley *et al.* (1977) determined the effect of RDX on midge larvae, pupae and adult survival and percent emergence. The most sensitive phase appears to be the larvae (see Table I-21). Survival of midge larvae was significantly reduced at RDX levels of 1.3 ppm. However the data from the study are inconsistent and the results do not appear to be dose related. Therefore in order to place any confidence in the data, the study should be repeated.

Bioconcentration of RDX by aquatic invertebrates was determined by Liu and Bailey (1977). Their results are presented in Table I-22. The bioconcentration factors are low. However, the data were not collected at steady state and are questionable.

f. Microorganisms

No information is available on the toxicity of TAX to microorganisms. Osmon and Klausmeier (1973) indicated that microorganisms could grow on complex media saturated with RDX.

5. Effects on Plants

a. Phytotoxicity

No information is available on the toxicity of TAX to plants. However, RDX should have a similar toxicity. The data in Table I-23 show the change in cell density versus RDX levels for four species of algae. *Selenastrum capricornutum* was the most sensitive specie tested, with a 29% reduction in cell density (Bentley *et al.*, 1977). Sullivan *et al.* (1979) statistically determined, using a one-way ANOVA, that the cell density reduction in *S. capricornutum* was significant at the 95% level. No effect levels of RDX using cell density were (Sullivan *et al.*, 1979):

Microcystis aeruginosa	10 ppm
Anabaena flos-aquae	.32 ppm
Selenastrum capricornutum	<.32 ppm
Navicula pelliculosa	3.2 ppm

The chlorophyll content of algae, Table I-24, was also reduced when the algae were exposed to RDX. Sullivan *et al.* (1979) statistically determined the significance of the reduction in chlorophyll levels. Using a one-way ANOVA they found the reductions in chlorophyll were only significant for the algae *S. capricornutum* at RDX levels of 0.32 ppm.

Table I-21. Mean (Standard Deviation) Percent Survival of the Midges (*Chironomus tentans*) Larvae[a], Pupae[b], Adults[b] and Percent Emergence of Adults[b] After Continuous Exposure to RDX (Bentley *et al.*, 1977)

Measured Concentration (mg/l)	Percent Survival[c]			Percent Emergence[c]
	Larvae	Pupae	Adults	
Control	52(18)	89(9)	85(8)	92(9)
1.3[d]	30(22)*	72(23)	33(2)*	100(0)
2.2	36(4)*	95(4)	70(21)	50(10)*
4.0[d]	25(11)*	87(4)	53(33)*	92(7)
10[d]	38(7)*	90(8)	78(12)	92(10)
21	38(12)*	89(11)	67(13)	77(33)

[a]Larvae survival determined after 19 days of exposure.

[b]Pupae and adult survival and percent emergence determined after 34 days of exposure.

[c]Each value represents the mean of four replicates.

[d]Second generation exposure initiated with control eggs.

*Significant at the 95% level.

Table I-22. Bioconcentration of RDX in Aquatic Invertebrates

Organism	Levels of RDX (mg/kg) Water	Tissue	Bioconcentration Factor	Reference
Water Flea *(Daphnia magna)*	0.31	0.52	1.7	Liu and Bailey (1977)
Oligochaete *(Lumbriculus variegatus)*	0.31	0.94	3.0	"

Table I-23. Percent Change[a] in the Cell Density[b] of Algea After 96 Hours Exposure to RDX (Bentley *et al.*,1977)

Nominal RDX Concentration (mg/1)	*Selenastrum capricornutum*	*Microcystis aeruginosa*	*Anabeana flos-aquae*	*Navicula pelliculosa*
0.32	-2	0	0	0
1.0	-5	-1	0	0
3.2	-17	0	0	-2
10	-23	-7	-4	-8
32	-38	-18	-14	-17

[a]Percent change is relative to control cultures.

[b]Determined by ceil counts for all species except *Anabeana flos-aquae* which was determined by optical density.

Table I-24. Percent Change[a] in the Chlorophyll *a* Content of Algae After 96 Hours Exposure to RDX (Bentley *et al.*, 1977)

Nominal RDX Concentration (mg/l)	*Selenastrum capricornutum*	*Microcystis aeruginosa*	*Anabeana flos-aquae*	*Navicula pelliculosa*
0.32	-3	0	0	0
1.0	-16	-3	0	-3
3.2	-17	-1	-3	0
10	-26	-11	-6	-9
32	-22	-21	-17	-23

[a]Percent change is relative to control cultures.

b. Bioaccumulation

Liu and Bailey (1977) found that the algae had a bioaccumulation factor of 108 for RDX. However, this was not at a steady state level. If the bioaccumulation of TAX parallels that of RDX, the concentration of TAX by aquatic plants should be low.

c. Degradation

No information is available on the degradation of TAX or RDX by plants.

F. Regulations and Standards

There are no regulations or standards for TAX in the United States. Bentley *et al.* (1977) and Sullivan *et al.* (1977) calculated water quality criterion for RDX exposure to aquatic organisms. Using a 0.01 application factor, Bentley *et al.* (1977) calculated a 0.35 ppm criterion.

Using the method described by EPA (Federal Register, 1979a), Sullivan *et al.* (1979) calculated a maximum allowable concentration of 1.0 ppm and a 0.3 ppm 24-hour average concentration.

G. Evaluation and Comments

Very little information exists on TAX. Although it is likely that this compound behaves like RDX in the environment, no data are available to support this assumption. RDX is moderately toxic to mammals and aquatic organisms. LD50's for mammals range from 18 to 300 mg/kg depending on the route of administration and the physical form of the RDX. The LC50's for fish are in the 4 to 6.5 ppm range. Aquatic invertebrates vary in their sensitivity to RDX. The midge is reported sensitive to concentrations above 1.3 ppm whereas *Daphnia magna* can tolerate concentrations of 20 ppm RDX with no apparent effects.

TAX is estimated to be present in the effluents of Holston AAP at 60 - 90% of the RDX levels. At full mobilization over 1 million lb of TAX per year would be released into the Holston River. Thus TAX could be a major pollution problem at Holston AAP.

Because so little information is known about TAX, the following studies are recommended in order to fill in the information gaps.

- Laboratory studies should be conducted to further define the solubility properties and aqueous chemistry of TAX. These studies should include solubility determination of TAX in aqueous medium under different pH conditions. Reactivity and potential decomposition of TAX as a function of pH and anions should also be determined. The aqueous solubility and reactivity of TAX in the presence of small amounts of organics such as acetone, cyclohexanone and formaldehyde also requires further study.

- Further sampling and analysis of Holston AAP effluents, the Holston River and river sediment are needed to verify existing data. However, before these studies are undertaken, the extraction and analytical methodology requires further investigation. This investigation should determine the optimum methods for sample storage, extraction and analysis in order that repeatable and reliable data may be obtained. Reliable analytical standards should also be prepared.

- No toxicological data exists for TAX. Therefore, the following studies are recommended:

    1. Acute mammalian studies should be conducted including i.v. injection of a TAX/DMSO solution in mice for comparison with RDX. An oral feeding study with mice or rats should also be undertaken. If these studies show that TAX is highly toxic, chronic toxicological studies may be warranted.

2. Acute and chronic aquatic toxicity studies should be conducted with species of fish and invertebrates found in the Holston River.

3. Bioaccumulation and biodegradation studies with TAX in aquatic organisms and microorganisms should be conducted. These studies will help determine the environmental fate of and potential hazards from this compound.

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PROBLEM DEFINITION STUDY ON
SEX
(1-ACETYLOCTAHYDRO-3,5,7-TRINITRO-1,3,5,7-TETRAZOCINE)

SUMMARY

SEX (1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine) is a by-product of RDX/HMX manufacture. The only current United States manufacturer of RDX and HMX is Holston Army Ammunition Plant, located in Kingsport, Tennessee. SEX is present in the wastewaters from Holston AAP at levels ranging from 50 to 90% of the HMX content. Current estimated discharges of this compound are between 14,000 and 28,000 lb/year. At full mobilization production of RDX and HMX, estimates of the quantity of SEX that would be discharged are between 0.4 and 0.8 million lb/year.

Only limited information is available on the physical and chemical properties of SEX. No information is available on the toxicological or environmental properties of this compound. Thus, the toxicological or environmental hazards of this compound must be inferred from the available information on HMX. HMX has an i.v. LD50 of 28.2 mg/kg to guinea pigs and a low toxicity to aquatic organisms. The presence of the $>N-\overset{O}{\overset{\|}{C}}-CH_3$ group in SEX is expected to increase its biological activity. However, the extent to which the presence of this functional group will alter the toxicological properties of SEX over those of HMX is not predictable.

In view of the limited information available on SEX, the following studies are recommended in order to fill in information gaps:

- further definition of solubility limits and reactivity of SEX in aqueous solutions
- additional sampling and analysis at Holston AAP to verify previous data
- an acute mammalian toxicological study
- acute and chronic aquatic toxicological studies
- bioaccumulation and biodegradation studies

FOREWORD

A. Study Goals

This report presents the results of an evaluation of the available information on he xicological and environmental hazards of SEX (1-acetyl-octahydro-3,5,7-trinitro-1,3,5,7-tetrazocine). SEX is a by-product of RDX and HMX manufacture at Holston AAP. This compound is found in the waste-waters from the manufacturing process. Holston AAP is the only source from which entry of SEX into the environment occurs in the United States. This evaluation of the toxicological and environmental hazard of SEX was under-taken in order to aid the Army in identification of research needs and in recommendations of environmental criteria for this compound.

B. Study Methodology

The methodology utilized to gather information for this report included a detailed search of the literature and numerous personal contacts. During the literature search, the following sources were reviewed for pertinent information on SEX:

- Chemical Abstracts	1940 - present
- Biological Abstracts	1950 - present
- Excerpta Medica	1950 - present
- TOXLINE	1965 - present
- National Technical Information Service	1964 - present
- Defense Documentation Center	1958 - present
- COMPENDEX	1970 - present

The search of the literature revealed very little information on SEX.

Personal contacts were made with Army Ammunition Plant personnel and Army and civilian researchers. The specific contacts made and results are presented below:

1. Manufacturers of HMX

a. Holston AAP Personal Contacts

Mr. Harold Shell and Captain Morris were contacted October 2, 1978, for information on SEX. They reported no toxicological data or observed health effects. On January 17, 1979, a trip was made to Holston AAP. The meeting was held with Mr. Bob Hash, Mr. Sam Wright and Mr. Buck Rogers. References on the chemical properties of SEX were received and we were told of recent research on SEX. Mr. Bob Hash indicated that some recent effluent data on SEX had been collected. On January 18, 1979 a letter was written to Mr Mike Mills requesting the effluent data and other information. The available information was received February 26, 1979.

b. Foreign

Only one manufacturer of HMX was listed in the 1978 SRI Chemical Producers Directory of Western Europe. In Belgium PRBsa was contacted and supplied us with references of toxicological studies concerning RDX and HMX.

2. Other Sources

Dr. Jay Abercrombie of the U.S. Army Chemical Systems Laboratory, Aberdeen Proving Ground, Md. was contacted September 12, 1978. He reported no information on SEX. Mr. J. Gareth Pearson of AMRDC, Fort Detrick, Md. was visited in September 1978. He had no toxicity data on SEX.

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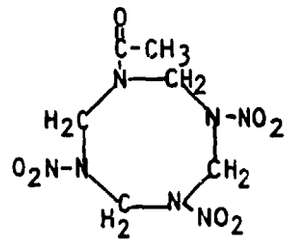
II. SEX (1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine)

A. Alternate Names

SEX (1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine) is a by-product of the RDX/HMX manufacturing process at Holston Army Ammunition Plant (AAP). This compound is formed during the nitrolysis of hexamine in acetic acid/acetic anhydride and is found in the wastewaters discharged from Holston AAP.

SEX has a molecular formula of $C_6H_{11}N_7O_7$ and molecular weight of 293.2. SEX has the following molecular structure:



Pertinent alternate names for SEX are listed below:

CAS Registry No.:	13980-00-02
CA Name (9CI):	1,3,5,7-tetrazocine, 1-acetyloctahydro-3,5,7-trinitro
Wiswesser Line Notation:	T8NC1 CNNW ENNW GNNWTJ
Synonyms:	SEX; 1-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocyclooctane

B. Physical and Chemical Properties

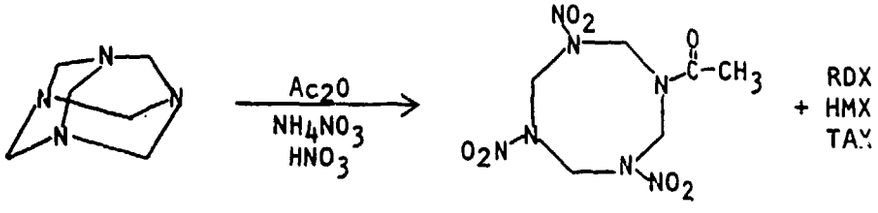
1. Physical Properties

Available physical properties of SEX are listed in Table I-1. The UV and infrared spectra of SEX are shown in Figures II-1 and II-2.

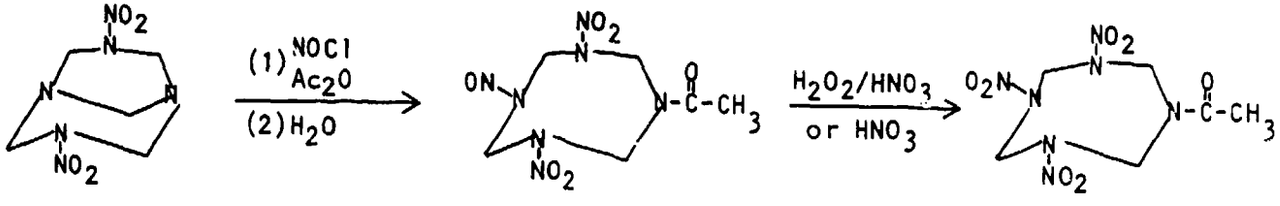
2. Chemical Properties

a. General Chemistry

SEX has been synthesized from hexamine as well as from some hexamine derivatives by reactions related to its occurrence as a by-product in the Bachmann synthesis of RDX. If hexamine is treated with nitric acid, acetic anhydride, and ammonium nitrate, RDX is formed. Upon neutralization, a 7.5% yield of SEX is also obtained (Aristoff *et al.*, 1949).



SEX can be prepared by treatment of 1,5-methylene-3,7-dinitro-1,3,5,7-tetrazocyclooctane with nitrosyl chloride and acetic anhydride which, upon hydrolysis, gives 1-acetyl-3,7-dinitro-5-nitroso-1,3,5,7-tetrazocyclooctane. This compound can be converted to SEX by treatment with hydrogen peroxide and nitric acid or by nitric acid alone (Bachmann and Deno, 1951).



1,5-diacetyl-3,7-dinitro-1,3,5,7-tetrazocyclooctane can also be converted to SEX upon treatment with 95% nitric acid.

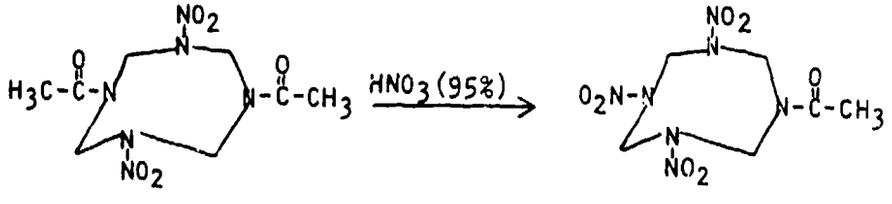


Table II-1. Physical Properties of SEX*

Physical Form @ 20°C:	solid
Color:	cream
Melting Point:	224.2 - 224.7°C
Volatility:	no data available
Vapor Pressure:	no data available
Specific Gravity:	no data available
Solubility: (no specific number available, in general slightly more soluble than HMX)	water - soluble in ppm level slightly soluble in pyridine, acetone and nitromethane almost insoluble in ethanol, acetic acid and ethyl ether
Octanol-Water Partition Coefficient:	No data available
X-ray Diffraction Spacings and Intensities:	6.95(s-), 6.62(s-), 6.08(vvf-), 4.74(vs), 4.44(m+), 4.01(vs), 3.71(f-), 3.54(s-), 3.44(m), 3.30(m), 3.18(m), 3.02(m), 2.92(m+), 2.73(f), 2.63(f), 2.54(vf+), 2.46(f), 2.41(f), 2.33(f), 2.26(f+) 2.22(f+), 2.10(vvf-), 2.03(f+), 1.98(vf+), 1.92(vf), 1.84(f), 1.82(f), 1.76(f-) 1.72(f-), 1.66(f-), 1.59(vvf), 1.53(vf-) 1.48(vvf), 1.44(vf-), 1.41(vvf+), 1.38(vvf), 1.35(vvf), 1.31(vvf), 1.28(vvf). (s = strong, m = medium, f = faint, v = very)
NMR spectrum has singlet resonances at:	2.24 ppm(3H,CH_3), 5.58(4H,CH_2) and 5.98 ppm(4H,CH_2)

*Aristoff *et al.*, 1949; Soldate and Noyes, 1947; Solomon *et al.*, 1973; Holston Defense Corporation, 1979.

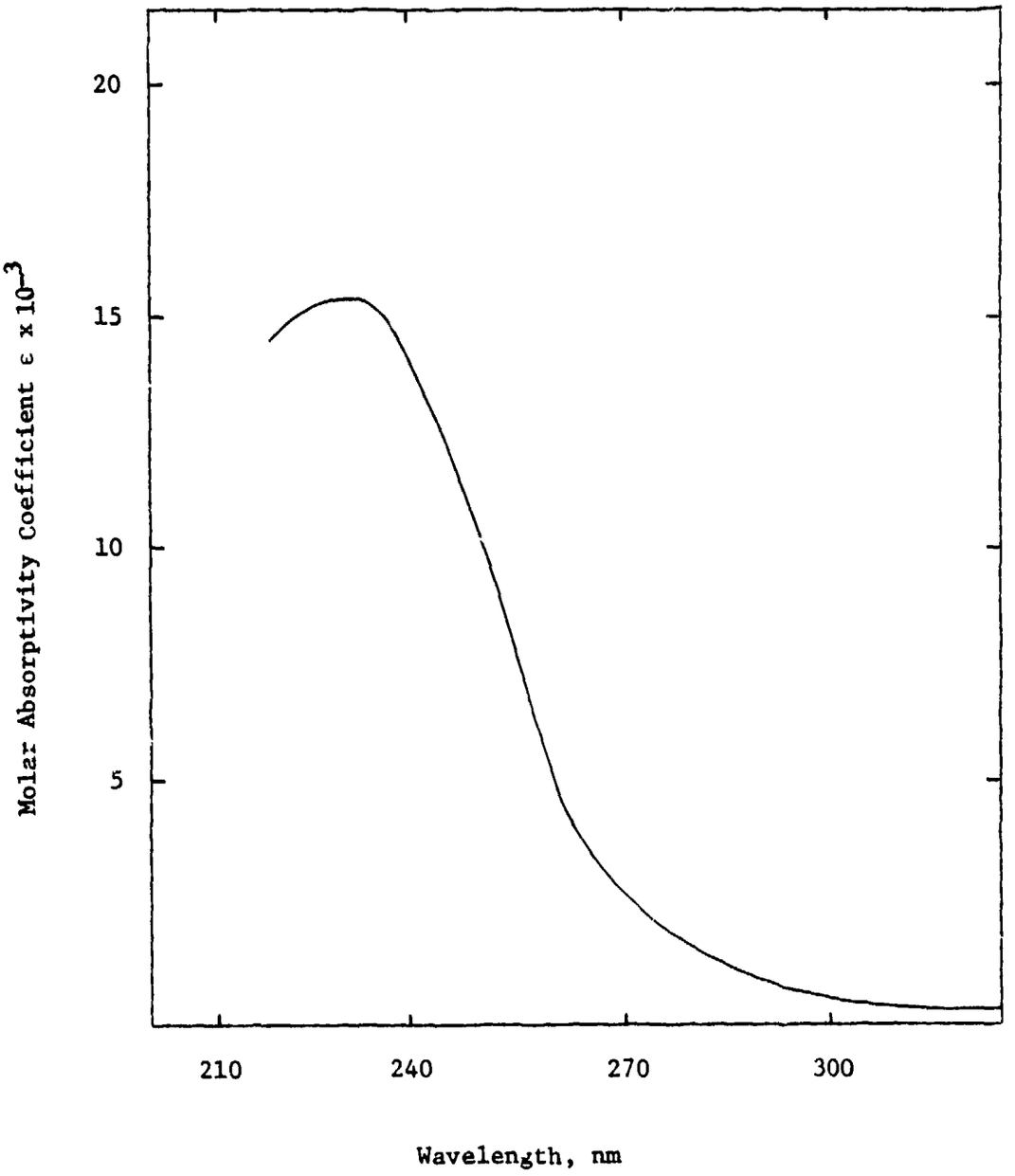


Figure II-1. Ultraviolet Spectrum of SEX (Schroeder *et al.*, 1951)

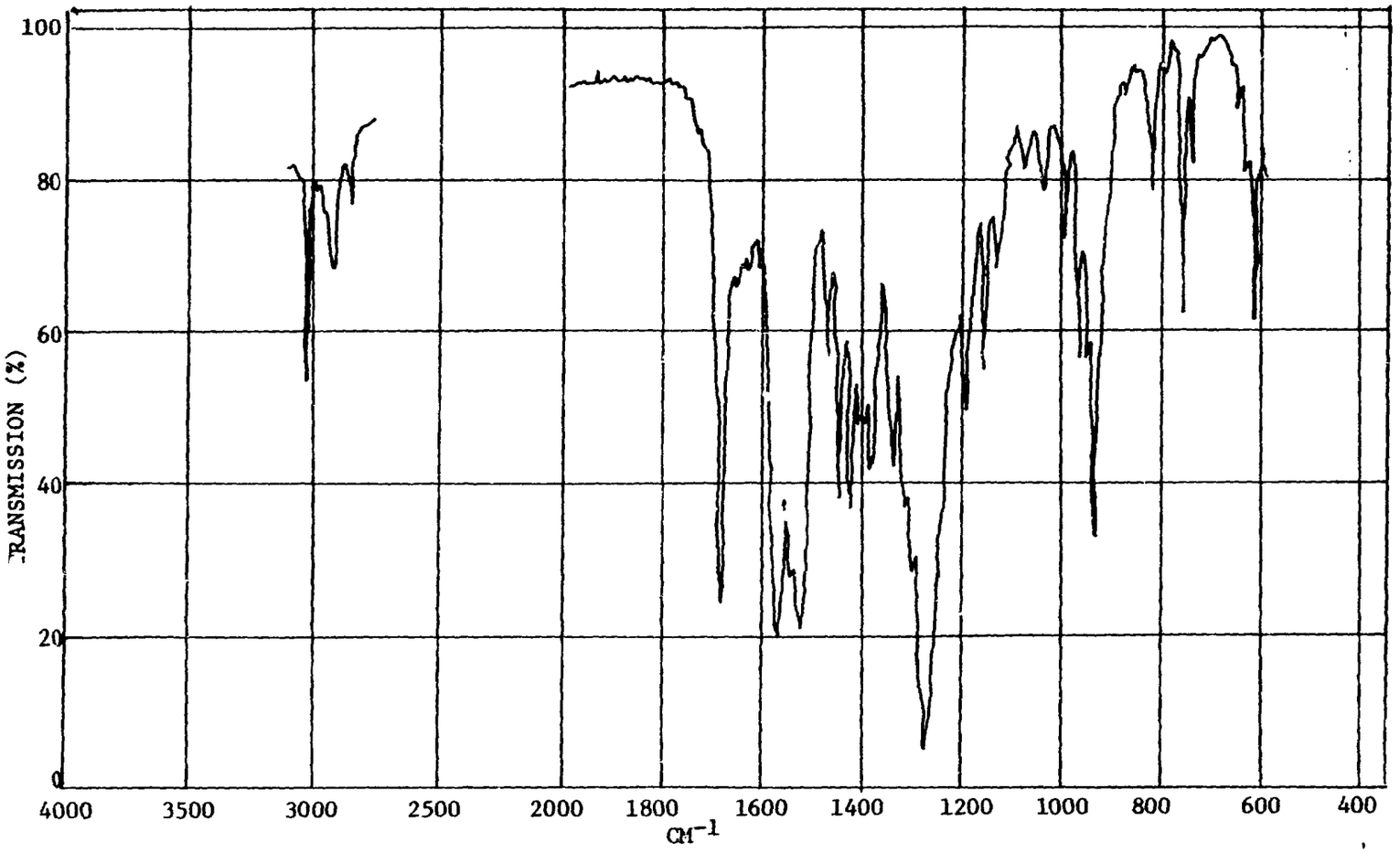
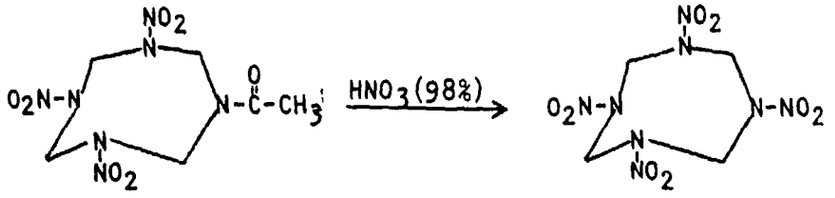
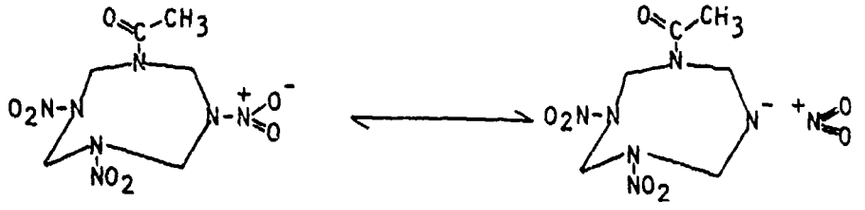


Figure II-2. Infrared Spectrum of SEX
(SEX-99.9% in KBr)
(Holston Defense Corporation, 1979)

SEX, like other nitramines, is subject to electrophilic substitution reactions. Treatment of SEX with 98% nitric acid yields HMX (Aristoff *et al.*, 1949).



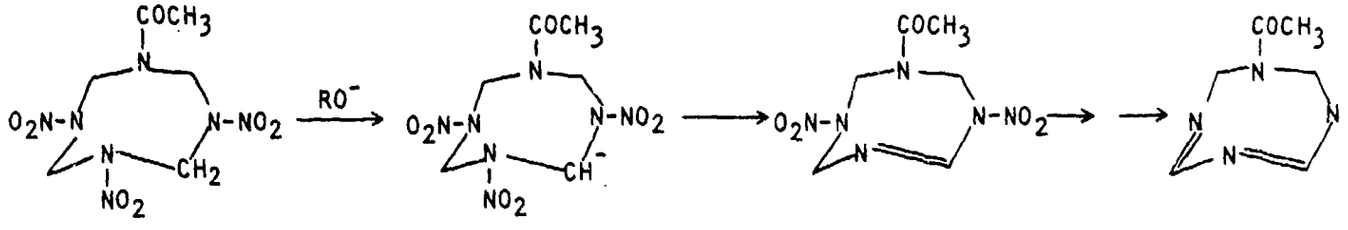
The nitrogen-nitrogen bonds are also very labile due to the resonance accumulation of negative charge at the ring nitrogens (Stals, 1969).



RDX will decompose in hot aqueous acid yielding NH_4^+ and HNO_3 among other products (Stals, 1969). The formation of NH_4^+ is indicative of an initial protonation of a ring nitrogen facilitated by the partial negative charge suggested by the above resonance structures. SEX would also be expected to decompose in hot aqueous acid forming NH_4^+, HNO_3 and HOAc.

SEX will detonate with a hammer blow (Aristoff *et al.*, 1949) yielding unidentified products. Similar decompositions of RDX and HMX are known to give N_2O, CH_2O, N_2, NO, CO_2, HCN and CO in order of decreasing yield (Stals, 1969).

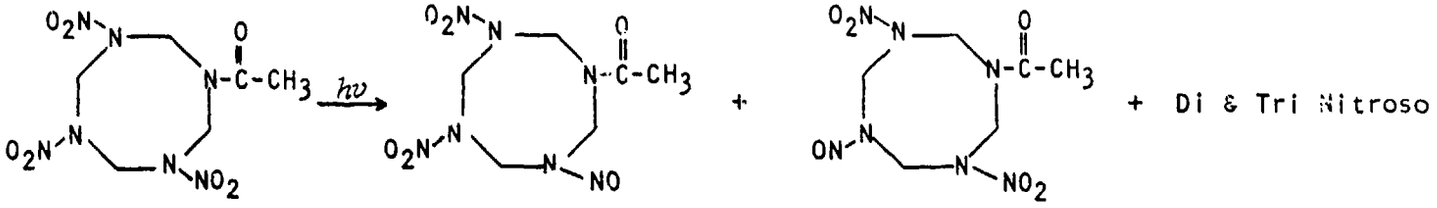
Secondary nitramines will undergo elimination reactions when treated with strong base. RDX, for example, gives s-triazine when treated with alkoxides (Stals, 1969). An analogous reaction for SEX would proceed as follows:



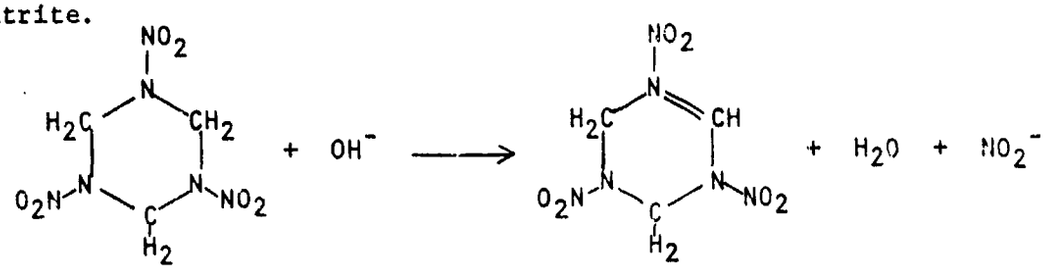
b. Environmental Reactions

The environmental fate of SEX has not been determined. However, recent studies on the environmental fate of RDX have been conducted. Photolysis of RDX has been carried out with both artificial irradiation and sunlight. Under artificial conditions, the mononitroso derivative of RDX was shown to be the major photoproduct (Kubose and Hoffsomer, 977; Sikka *et al.*, 1978) Nitrite, nitrate, formaldehyde and nitrogen are also formed. The extent of photolysis to the mononitroso derivative is dependent on pH and nitrite ion concentration. Low pH and high nitrite concentrations inhibit the initial photolysis. Dissolved oxygen has no effect on the photochemistry. The quantum yields for RDX photolysis in a pH 8.08 borate buffer is 0.69 (Sikka *et al.*, 1978). Photolysis of RDX in tap water in natural sunlight also occurs. The half life for sunlight photolysis of RDX is estimated at 9-13 hours (Sikka *et al.*, 1978).

A similar photolysis reaction for SEX would be expected yielding the following products:



Hydrolysis of SEX in the environment is also likely if conditions are severe enough. However, no studies have been performed with this compound. Hydrolysis of RDX has been reported. At pH 2-7 virtually no hydrolysis occurs within 12 days (Sikka *et al.*, 1978). At pH 9.07 and 31°C, ∿ 27% of the RDX is hydrolyzed in 21 days. In studies of basic hydrolysis of RDX, Hoffsomer *et al.* (1977) showed that the reaction is a concerted E2 elimination process with proton abstraction by hydroxide ion and simultaneous loss of nitrite.



A similar reaction would be expected for SEX. However, the reaction should stop short of complete conjugation. The acetyl group is not a good leaving group and extremely severe conditions are required to hydrolyze the amide linkage.

C. Monitoring and Analysis

1. Analytical Methods

Analysis for SEX has been reported by a semi-quantitative column chromatography technique (Malmberg *et al.*, 1953). SEX was chromatographed on silicic acid/Celite 535(2:1). Partial separations (SEX was not completely separated from TAX) were obtained using 50% ethyl acetate in benzene as a mobile phase. Quantitative removal of SEX from the adsorbent was about 91% efficient using a 1:1 acetone-ether solution. The colorless chromatographic zones were visualized using streak reagents on the extruded column which consisted of zinc dust, benzene, sulfanilic acid and 1-naphthylamine. As little as 0.01 mg of the nitramine could be detected.

Bell and Dunstan (1966) have studied the use of thin layer chromatography for the analysis of SEX and other nitroamines. Chromatograms were run on Silica gel G using benzene/nitromethane, chloroform/nitromethane, petroleum ether/acetone and ether/acetone solvent systems. Visualization was accomplished by spraying with diphenylamine and irradiating with ultraviolet light. Harthon (1961) also used thin layer chromatography to analyse nitramine explosives. Petroleum ether/acetone was used as a solvent system on Silica gel G. Visualization was accomplished with diphenylamine and ultraviolet irradiation.

The best methodology for quantitative analysis of SEX is high performance liquid chromatography (HPLC). This method has been used by Holston Defense Corporation for analyses of effluent samples. Chromatograms were run using a Li Chrosorb Si 60 column in isocratic mode with a methanol, acetonitrile, chloroform, iso-octane (1:2:3:14) solvent system. Detection was accomplished with a 245 nm UV detector. The lower limit of detection was <.0007 ppm.

2. Monitoring

In 1977, Holston Defense Corporation sampled three effluent streams and the Holston River to determine the levels of SEX and TAX in these streams. The four sampling locations were:

- N-3 - manhole below Building N-3 which carries effluents from the G (RDX recrystallization), H, I, J, K, M and N (RDX, HMX incorporation) Buildings on line 1 through 5.

- N-6 - manhole below Building N-6 which carries process effluents from Buildings D6 (nitration), E6 (acids removal and explosives wash) and G6 (recrystallization).

- T-2 - manhole below T-2 (acid area) which carries process effluents from Buildings C3, C5 (reagent preparation), B9, B11 (primary distillation and ammonia recovery), D3, D5 (nitration), E3 and E4 (acids removal and explosives wash).

- Holston River at the area B boundary

The levels of SEX, TAX, RDX, and HMX in these streams are presented in Table II-2. Because sample storage caused a wide variation in analytical results, the analyses only for those samples which were immediately extracted and stored in acetonitrile are presented.

From the data in the table, it appears that SEX is entering the environment from the nitration buildings (D-Buildings), the acids removal and explosives wash (E-Buildings), recrystallization (G-Buildings), dewatering (H-Buildings) and the incorporation Buildings (I, J, K, L, M). However, the major quantities of SEX entering the environment are from the dewatering and incorporation steps.

The average concentrations of RDX, HMX, TAX, and SEX at the four sampling points are presented below:

Sample Point	RDX(mg/l)	HMX(mg/l)	TAX(mg/l)	SEX(mg/l)
N-3	5.5	2.6	4.8	2.3
N-6	4.5	1.6	2.6	0.8
T-2	0.3	0.12	0.02	0
River	0.01	0.01	0.004	0

In the effluent from the dewatering and incorporation steps, SEX is presented at levels between 50 to 90% of the HMX or 20 to 40% of the RDX present. At full mobilization, ~208 million pounds of RDX is produced yearly. If 1% of the RDX product is lost in the effluents, then Holston AAP could discharge as much as 2.1 million pounds of RDX per year. Discharges of SEX could be 0.4 to 0.8 million lb/year if the 20 to 40% ratio of SEX/RDX is valid.

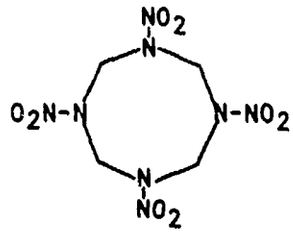
Table II-2. Analysis of Waste Streams for RDX, HMX, TAX and SEX (Holston Defense Corporation, 1977).

Date	Sample Point	RDX (mg/l)	TAX (mg/l)	HMX (mg/l)	SEX (mg/l)
5/17-18/77	N-3	10.0	8.7	2.2	2.2
	N-6	6.3	6.3	1.3	0.2
	T-2	0.2	*	0.04	*
	River	0.01	0.003	*	*
5/24-25/77	N-3	7.2	16.9	2.4	4.0
	N-6	2.2	2.2	1.3	1.0
	T-2	0.4	*	0.6	*
	River	0.007	0.003	0.03	0.001
5/31/77-6/1/77	N-3	2.4	*	4.5	4.8
	N-6	7.0	3.1	2.1	0.6
	T-2	0.4	*	0.07	*
	River	0.006	*	0.006	*
6/6-7/77	N-3	4.3	*	1.5	1.9
	N-6	5.6	5.6	1.7	1.5
	T-2	0.4	0.007	0.1	0.001
	River	0.009	0.003	0.006	0.0007
6/8-9/77	N-3	2.1	2.2	0.3	0.8
	N-6	1.0	1.0	0.4	0.3
	T-2	0.1	0.005	0.03	*
	River	0.0005	0.01	0.0009	*
6/13-14/77	N-3	10.4	5.8	2.9	2.2
	N-6	3.9	0.007	2.4	1.3
	T-2	0.2	0.04	*	*
	River	0.02	0.001	0.01	*
6/17-19/77	N-3	1.8	0.007	4.7	0.5
	N-6	5.3	3.4	2.3	0.07
	T-2	0.3	0.1	*	*
	River	0.02	0.005	0.03	*

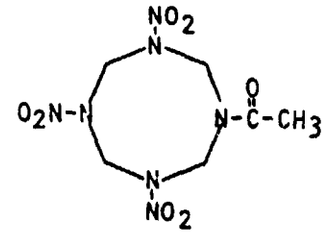
*not detected

D. Health Effects

No information on the health effects of SEX was found in the literature search. The information presented herein is based on extrapolation of data from the HMX parent compound. The structures of these two compounds are shown below.



HMX



SEX

The presence of the amide group, $>N-\overset{O}{\overset{\|}{C}}-CH_3$, is expected to increase the biological activity of SEX over that of HMX. The extent and the implication of this expected increase in biological activity are not predictable.

The literature available on the effects of HMX (and by inference, SEX) consists almost solely of a monograph by McNamara *et al.* (1974). The report is a compendium of various experiments done on mice, guinea pigs, and dogs on the toxicity of both RDX and HMX in the solvents DMSO, cyclohexanone, and acetone.

1. Biology

a. Absorption

Absorption of SEX or HMX can occur through gastrointestinal or respiratory tracts. The efficiencies of absorption of SEX or HMX by these routes have not been studied. However, a major study has been conducted to evaluate the skin absorption of HMX in different solvents (McNamara *et al.*, 1974). In this study, HMX dissolved in DMSO, cyclohexanone or acetone was applied in acute and subacute doses to the shaved skin of rabbits, guinea pigs and beagle dogs.

In rabbits, percutaneous application of HMX in various solvents was performed according to the following specifications (McNamara *et al.*, 1974). For acute studies, a solution of HMX in DMSO (33% w/v), and suspensions of HMX in cyclohexanone (75% w/v) and acetone (5.4% w/v) were used. Eighteen test animals and nine control were divided into three groups each. Within

each test group, the six test animals received a 1 ml dose of HMX in solvent, and the three control animals received a 1 ml dose of corresponding solvent. After all applications, a polyethylene sleeve was taped to each rabbit's back. The sleeve was removed after 24 hours and the skin was immediately examined for irritation. Observation of the rabbits continued for 30 days.

Blood samples were drawn from each rabbit in order to monitor any possible biochemical changes which may have resulted from HMX applications. Parameters analyzed included: red and white blood cell count, hematocrit, hemoglobin, alkaline phosphatase, serum glutamic oxalacetic transaminase, blood urea nitrogen, creatinine, sodium, potassium, chloride, and CO_2. After one hour, 3 days, and 30 days, two rabbits from each dose group and one from each solvent group were sacrificed for pathological examinations. These acute studies showed that no evidence of dermatitis (gross or microscopic) or change in blood constituents could be attributed either to the solvents or dissolved HMX.

In subacute studies, doses of 0.1 or 1 ml of HMX in DMSO (33% w/v), cyclohexanone (2.5% w/v) or acetone (2.0% w/v) were applied to the backs of rabbits 5 days/week for 4 weeks (McNamara *et al.*, 1974). Six rabbits were exposed to each mixture and volume, and control animals in groups of three received both volumes of solvent alone. Each 1.0 ml dose of HMX contained 165 mg/kg in DMSO, 12.5 mg/kg in cyclohexanone, and 10.0 mg/kg in acetone. Gross observation and blood analyses were similar to acute studies. Two rabbits from each dose group and one from each solvent group were sacrificed on the 7th, 14th and 28th day.

During the course of the study, rabbits receiving only the 1 ml dose of crude DMSO had slight skin desquamation in the second week; none of the other solvents had any effect. Repeated doses of 1.0 ml of HMX in DMSO produced mild desquamation at the end of the first week. In addition, three deaths occurred; one after two doses (330 mg/kg), one after six doses (990 mg/kg), and one after 20 doses (3300 mg/kg). No details concerning the deaths of these animals were given. For all other mixtures and volumes of HMX, no gross evidence of dermatitis or systemic toxicity was observed. Pathological examination of both dead and sacrificed animals revealed no lesions other than at the site of application. Microscopic examination showed minimal dermatitis with HMX dissolved in acetone and cyclohexanone, and normal controls.

In the single and repeated doses tests, six rabbits were used for each experimental point, and three rabbits were used for each of three controls. Although blood samples were drawn from each rabbit, there was no indication of when during the experiment the samples were taken. Animals were also sacrificed at various times during the study for pathological examination. Skin irritations produced by various doses were discussed and presented in tabular form, but the effects of solvents alone were only discussed in the text. For ease of comparison, all of these cases should have

been tabulated together. The text states that three rabbits died at various times during the repeated applications study. Although no lesions were found in these animals upon necropsy, a description of the circumstances surrounding their deaths, *e.g.*, behavior-symptomatology, should have been included. The authors wisely drew no conclusions from these studies. More animals would have to be tested in order to present statistically significant conclusions.

Guinea pigs in groups of two received four acute percutaneous doses of HMX in DMSO: 316, 510, 1000, 2000 mg/kg (McNamara *et al.*, 1974). None of the guinea pigs exposed to the 316 or 510 mg/kg doses showed any effects. However, slight erythema was observed with the 1000 and 2000 mg/kg doses. One subacute exposure consisting of three doses of 1000 mg/kg resulted in apprehension, appetite weight loss, and loss of normal skin color. No other details of this study were presented, in particular when the observations of skin effects were observed. Furthermore, although the results are presented in a table, no numbers are given. If the reader is to assume that the effects noted occurred in all of the animals, this should have been stated. Although DMSO alone was applied and produced no effects, this is a nebulous result since the volume applied was much smaller than that applied with HMX. This experiment was more characteristic of a pilot study than a full investigation.

Beagle dogs also were exposed to various solutions of HMX by the percutaneous route (McNamara *et al.*, 1974). Three types of exposure were used as outlined in Table II-3. The blood pressure, respiratory and heart rates, EKG and EEG were measured at the time of exposure and weekly thereafter for four weeks (except for blood pressure which was measured again only on the fourth week). Examinations for hyperreflexia were also made. Results of the topical application of up to 289 mg/kg HMX for acute exposure showed no consistent change in any physiologic parameters. Subacute exposures were up to 289 mg/kg/day for five days/week for four weeks. Again there were no consistent changes in the parameters measured, except during the second or third week, some dogs exhibited slight erythema and desquamation with application of DMSO alone or in combination with HMX. Furthermore, there were no consistent changes in the physiologic parameters after a massive chronic exposure of 480 mg/kg for three consecutive days.

From the absence of consistent changes in any physiologic parameters studied in the above mentioned animals, it is evident that topical application of HMX and probably SEX, does not result in efficient skin penetration even when dissolved in penetrating agents such as DMSO. However, the data are limited to small numbers of animals and must be interpreted with caution. Some penetration does occur in DMSO (resulting in production of physiologic effects), but only at levels 10 to 1000 times the intraveneous LD50 (see Section D.1.c.).

Table II-3. Percutaneous Administration of HMX in Various Solvents to Dogs (McNamara *et al.*, 1974)

Type of Dose	Solution	No. of Days	Dose mg/kg/day	Total Dose mg/kg	Results
Acute	33% HMX in DMSO	1	289.0	289	No consistent changes in parameters measured.
Acute	2% HMX in Acetone	1	17.5	17.5	" "
Acute	2.5% HMX in Cyclohexanone	1	21.9	21.9	" "
Subacute 5 days/wk for 4 weeks	33% HMX in DMSO	20	289.0	578.0	No consistent changes in parameters measured. Some erythema during 2nd and 3rd week.
Subacute 5 days/week for 4 weeks	2% HMX in acetone	20	17.5	350	No consistent changes in parameters measured.
Subacute 5 days/week for 4 weeks	2.5% HMX in Cyclohexanone	20	21.9	438	" "
Massive Subacute 3 consecutive days	33% HMX in DMSO	3	480.0	1440	No consistent changes in parameters measured.

b. Transport, Metabolism and Elimination

No information was found on the transport, metabolism or elimination of HMX or SEX in mammals.

c. Pharmacology

In order to obtain concentrations of HMX high enough to produce effects on physiologic parameters, unanesthetized dogs were given intravaneous (i.v.) doses of HMX in solution (McNamara *et al.*, 1974). The pharmacologic action of HMX was determined by measuring its effects on the central nervous system (CNS) of the dogs.

When single dose of 40 mg HMX/kg in DMSO was administered i.v., it produced severe cardiovascular collapse in all four dogs tested. The cardiovascular collapse was accompanied by a narrow pulse pressure, bradycardia and respiratory changes. The EEG showed high-voltage, low frequency discharge. Two animals died in three minutes, the other two at about 14 hours. Administration of 40 mg/kg of HMX in DMSO in two separate doses of 20 mg/kg to two animals produced severe cardiovascular depression; one animal died in one minute. The second dog recovered from the cardiovascular depression and showed EEG hyperactivity. This animal was extremely sensitive to photic and tactile stimuli; it died at about 14 hours after injection. No autopsy results on the dead animals were reported.

When two dogs were given a 20 mg/kg dose of HMS in DMSO i.v., only minimal changes occurred in one of the dogs tested. This dog had emesis after 15 minutes, then stabilized for two hours, at which time hyperreflexia to vibratory and light stimuli occurred. However, visual perception and lid corneal reflexes were normal. At five hours post injection, this dog became markedly hyperactive and suffered convulsive seizures. The animal did not recover until five days after exposure.

Administration of 2.5 mg HMX/kg in acetone (2% w/v) produced transient hypotension in a dog. During the 12-to-40 minute post injection period, the EGG showed a sleep wave pattern. However, the animal could be easily aroused. No irregularities in EEG pattern could be observed the following day. A larger dose, 6.75 mg/kg HMX in acetone (5.4% w/v) produced a decrease in blood pressure together with high-voltage, low-frequency EEG discharges. The animal appeared normal the next day. One dog was given 6.75 mg/kg (5.4%) HMX-acetone after receiving a prior injection of the 2.5 mg/kg (2%) solution. The second dose produced a drop in blood pressure, cardiac arrest, and depressed respiration rate, but the EEG was not affected. At various times the dog vomited and slept, but could be aroused; the animal appeared normal the next day. Administration of both 1.55 and 3.1 mg/kg in cyclohexanone produced cardiovascular collapse, onset of a coma-like state, and elevation of the pain threshold. No convulsions were noted and both dogs recovered completely in two to three days.

As controls, dogs received injections of each of the three solvents. In three dogs, cyclohexanone produced immediate cardiovascular collapse, with cardiac arrest lasting about 10 seconds. These animals also exhibited narrow pulse pressure and EEG changes and were either in a comatose or semicomatose state; recovery was complete by 120 minutes post injection. In four dogs, acetone produced a decrease in blood pressure up to 60 seconds; bradycardia, followed by tachycardia, was also observed (3 of 4 dogs). With DMSO, two dogs showed no changes, while two had decreased blood pressure, but only for 5-10 seconds. Compensatory tachycardia occurred with decreased blood pressure and recovery was prompt.

The pharmacologic activity of HMX leads one to assume that this compound acts similarly to a "nitrite-like" compound. Unlike nitrites, HMX did not produce the chocolate-colored blood that is indicative of methemoglobin formation. The i.v. administration of HMX produces an initial circulatory collapse. Depending on level administered, death can be immediate. Recovery from circulatory collapse is followed by CNS disturbances which require several days for recovery. Considerable work in the area of pharmacology remains to be done with larger numbers of animals so that definite trends can be established (McNamara *et al.*, 1974).

2. Effects of Human Exposure

a. Epidemiology

No epidemiological information was found on SEX or HMX.

b. Occupational Exposure

Only one occupational exposure study was reported in the literature (Hathaway and Buck, 1977). This study attempted to evaluate the effects of exposures of RDX of up to 1.5 mg/m^3 on workers at any Army Munitions Plant. HMX and SEX were presumably also present in the air; however, levels were not measured. No difference in the number of abnormalities in the hematologic, hepatic or renal systems or the presence of autoimmune disease were found among these workers as compared to unexposed controls.

3. Effects on Experimental Animals

a. Acute Toxicity

In mice, the intravenous LD50 for HMX was 28.9 mg/kg. For guinea pigs, LD50 for HMX in DMSO was found to be 28.2 mg/kg. In both instances, convulsions occurred and death was within five minutes after

injection (McNamara *et al.*, 1974). The percutaneous LD50 in guinea pigs is uncertain since in one experiment death occurred after a single dose of about 500 mg/kg, while in another experiment, no deaths occurred at doses from 316 to 2000 mg/kg.

For the mouse experiment, there were no controls, *i.e.*, no mice were injected with DMSO alone. However, six mice each were used for the five different doses of HMX. The determination of the LD50 for these mice is probably accurate based on the available data, but should not be given much credence since no controls were performed.

Guinea pigs received four different doses of HMX, two animals per dose. Proper controls were performed and the response to these injections indicated no effects due to solvent alone. Results for the LD50 are quoted as being at the 95% confidence level. For an experiment involving only 8 animals, it is presumptuous to present an LD50 in this manner. The results are valid for 8 animals, but more data are needed if a 95% confidence level is to be placed on them.

I.v. injection of HMX/DMSO in dogs showed that 40 mg/kg was fatal to all animals. Lower doses of HMX in DMSO, acetone, and cyclohexanone produced various physiologic changes, but none of the animals died. (See Section D.1.c. for details).

b. Subacute Toxicity

In subacute studies in which HMX in DMSO, acetone or cyclohexanone were applied topically to dogs, doses used were 289.0, 17.5, and 21.9 mg/kg respectively. These doses were administered five days/week for four weeks; results showed no change in blood pressure, heart or respiration during this exposure. All animals responded normally to visual, corneal, and lid tests at all times (McNamara *et al.*, 1974).

A second subacute study on dogs involved percutaneous application of massive doses (480 mg/kg 33% HMX in DMSO) for three consecutive days. Results showed no consistent gross changes in the two dogs studied; they were held for two weeks after exposure and appeared normal (McNamara *et al.*, 1974).

In studies on rabbits in which either 0.1 or 1.0 ml of HMX in DMSO (33% w/v), in cyclohexanone (2.5%w/v), and in acetone (2.0%w/v) were applied topically, six rabbits died. All of the dead rabbits had been receiving 1.0 ml doses of HMX 33% in DMSO. Three rabbits died after the second dose (330 mg/kg), one after the fifth dose (825 mg/kg), one after the sixth dose (990 mg/kg), and one after the 20th dose (3300 mg/kg) (McNamara *et al.*, 1974).

After topical exposure to 0.5 ml of 33% HMX in DMSO, five of 12 guinea pigs died in 24 to 48 hours; four died after single doses of 405, 477, 507, and 546 mg/kg, and one after two doses of 1126 mg/kg. These deaths were apparently due to HMX poisoning since other animals receiving DMSO showed no toxic signs (McNamara *et al.*, 1974). No autopsy results were given. Guinea pigs receiving repeated 0.5 ml application of 2.5% HMX in acetone or 2.0% HMX in cyclohexanone showed no signs of toxicity or death. However, cyclohexanone alone and with HMX produced no skin irritation (McNamara *et al.*, 1974). The conclusion that HMX produced no sensitization is valid for the animals tested. Again, testing a larger number of animals would have produced more statistically significant results. Based on the deaths which resulted in this experiment, the authors calculate a lethal percutaneous dose for 70 kg/man. Since it was stipulated that this comparison is only valid if humans are toxicologically comparable to guinea pigs, the calculation can be given comparable merit.

The eyes of a group (218 animals) of guinea pigs were examined after they had received either cutaneous or intradermal application of HMX in three solvents three times a week for three weeks. Cutaneous application consisted of 0.5 ml of either 33% or 3.3% HMX in DMSO, 2.5% HMX in cyclohexanone, or 2.0% HMX in acetone; intradermal application consisted of 0.5 ml of 1:1 0.25% HMX in solvent/saline. Solvents alone were also administered by both routes. Cataracts developed in about 20% of the animals and the HMX did not appear to increase production of the cataracts (McNamara *et al.*, 1974). However, no statistical analysis of the results was presented. No other effects were described.

c. Chronic Toxicity

No studies have been conducted to determine the chronic toxicity of HMX or SEX.

d. Teratogenicity

No studies have been conducted to determine the teratogenic effects of SEX or HMX.

e. Mutagenicity

The Ames bacterial mutagenicity test was performed on Holston AAP wastewater effluents (Stilwell *et al.*, 1977). A set of five *Salmonella typhimurium* strains, TA-1535, TA-1537, TA-1538, TA-93 and TA-100 were used. The histidine deficient variant strains are used to detect frame shift reverse mutations (TA-1537, 1538 and 98) or base pair substitutions (TA-1535 and 100). A dose response curve ranging from 5 ml to 100 ml of test water was used for each strain. An index of relative mutagenicity was

used (i.e. test reversions/control reversions). The value of 1.0 indicated no mutagenic activity with 10 clearly indicating mutagenic activity. The highest wastewater index was 2.07 and was attributed to chance variation. Therefore, Stilwell *et al.* (1977) found no mutagenic properties in Holston AAP effluents.

f. Carcinogenicity

McNamara *et al.* (1974) carried out subacute percutaneous administrations of HMX in various solvents with rabbits. This study was described in detail in Section D.1.a. Administration of the HMX was five days per week for four weeks. Pathological examination of sacrificed animals showed no lesions except at the application site, but these animals had only been sacrificed immediately after receiving doses of HMX. However, there was no evaluation of possible long-term effects, *e.g.*, carcinogenesis.

g. Behavior-Symptomatology

Immediate symptoms of HMX intoxication in dogs are cardiovascular collapse. If recovery from the cardiovascular depression occurs, central nervous sytem disturbances are observed. These include EEG hyperactivity, convulsions, and increased sensitivity to photic, tactile and auditory stimuli (McNamara *et al.*, 1974)

h. Sensitivity

McNamara *et al.* (1974) tried to sensitize guinea pigs to various mixtures of HMX in DMSO, cyclohexanone, and acetone by both topical and intradermal routes; the procedures of Landsteiner and Jacobs (1935) and Landsteiner and Chase (1937, 1940 and 1941) were used. Test solutions consisted of 33% and 3.3% HMX in DMSO, 2.5% HMX in cyclohexanone and 2.0% in acetone. For topical application, 0.5 ml of test solution was used, but for intradermal injection, 0.005 ml of test solution was used. Initial experiments used 0.5 ml 33% HMX in DMSO, but deaths occurred after the first and second application (see Section D.3.b for details). When the topical sensitizing dose was reduced to 3.3% HMX in DMSO, no animals died. After the sensitization phase, in which each animal received three applications per week for three weeks, there followed a two-week rest period. Animals also received pure solvent according to the same schedule.

In the challenge phase, animals were exposed either i.d. or topically to single doses of test material at a predetermined maximum subeffective level. Although only one route was used to sensitize each animal, both routes were used to challenge. Results of these studies showed no evidence of skin sensitization from any combination of exposure to HMX in solvent or solvent alone.

E. Environmental Effects

1. Entry into the Environment

Wastewaters from the manufacture of HMX and RDX at Holston AAP are the only source of entry of SEX into the environment in the United States. These wastewaters currently flow either directly or indirectly into the Holston River. In 1977, Holston Defense Corporation sampled three effluent streams and the Holston River to determine the levels of SEX. These data were presented in Section C.2. In summary, the data showed the level of SEX in the effluent streams from the dewatering and incorporation steps to be between 50 and 90% of the HMX and 20 to 40% of the RDX present. Values ranged from near 0 in the Holston River to an average of 2.3 ppm in the effluent from the dewatering and incorporation steps.

2. Behavior in Soil and Water

a. Transport and Accumulation

There is very little information available on the behavior of SEX in the environment. Until specific information is available, predicting the movement and accumulation of SEX in the environment must be made from information available on RDX and HMX.

Barkley (1977) determined the solubility of HMX in water at 20°C to be 6.6 ppm. SEX with the substitution of an acetyl group, should have a slightly higher solubility.

Sikka *et al.* (1978) studied the adsorption of RDX on three sediment types. As shown in Table II-4, RDX was poorly adsorbed onto the sediment. However, Sikka *et al.* (1978) had a 1:100 adsorbent: solution ratio, while the EPA (Federal Register, 1979b) recommends a 1:5 ratio. Increasing the amount of sediment could increase the adsorption values for RDX onto the sediment.

Table II-4. Adsorption of RDX by Sediments
(Sikka *et al.*, 1978)

Sediment	Partition Coefficient (K_p)
Organic Muck	4.15
Clay Loam	3.06
Sandy Loam	0.80

Sullivan *et al.* (1977) reported an average level of HMX in Holston AAP effluents to be 0.87 ppm. They also measured RDX and HMX levels in the sediment of the Holston River and found neither compound above the 0.2 ppm detection limit.

The absence of RDX, HMX and probably SEX in the sediment of the Holston River near Holston AAP is probably due to scouring of the sediment (Sullivan *et al.*, 1977). This scouring occurs because of the wide fluctuation of water levels in the river due to the Ft. Patrick Henry Dam. As shown in Figure II-3, the water levels in the Holston River vary by 10 fold daily. Thus, any chemicals which accumulate in the sediment near Holston AAP are rapidly washed down the river when the dam gates are opened. However, it does not appear from the information available on RDX and HMX that the sediment is a "sink" for the explosive compounds and their byproducts.

b. Degradation

Sikka *et al.* (1978) studied the photolysis of RDX exposed to UV light around 230 nm. The tests were apparently conducted in distilled or tap water. The results indicated, Table II-5, that RDX was substantially degraded within five hours. Also nitrite and nitrate concentrations increased during the irradiation.

The removal of RDX or SEX in the environment through photolysis is doubtful. Wetzel (1975) stated that while distilled water absorbs small amounts of UV light, low concentrations of dissolved organic compounds absorb large amounts of UV light. The data in Table II-6 indicate an almost total absorption of UV, blue and green wavelengths in lakes with high dissolved organic levels. Sullivan *et al.* (1977) reported total organic carbon levels in the Holston River ranged from 3-330 mg of carbon/l. Total dissolved solids ranged from 62-576 mg/l in the river. The RDX or SEX present in the water would have to compete with other dissolved organic compounds for the UV light. In addition, within a water depth of 1 meter essentially all of the UV light would probably be absorbed.

The hydrolysis of RDX has been found to be a slow process. Sikka *et al.* (1978) found no hydrolysis of RDX, under acidic conditions, over a 12-day period. However, they found 27% of the RDX was hydrolyzed under basic conditions (Table II-7). The hydrolysis of SEX, because of the acetyl group, should be less complete than RDX or HMX.

Spanggord *et al.* (1979) stated that volatilization should not be a major factor in the environmental fate of RDX. Because SEX and RDX are solids at 20°C and have a high boiling point, it is likely that the volatilization rate of SEX is also low.

c. Background Concentrations

Background concentrations of SEX in natural areas are non-existent.

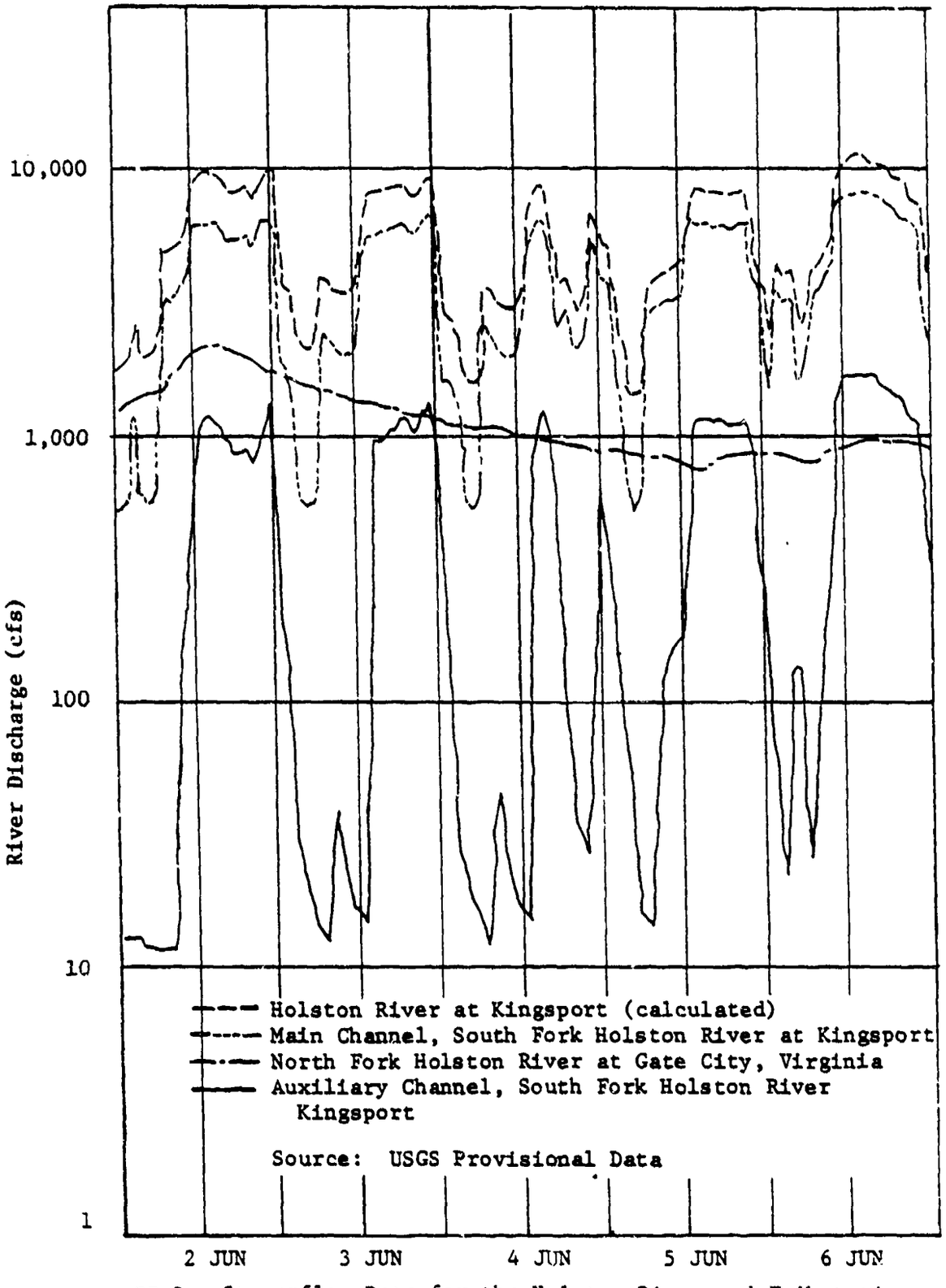


Figure II-3. Streamflow Data for the Holston River and Tributaries at Kingsport. June, 1975 Sampling Trip. (Sullivan *et al.*, 1977)

Table II-5 Photolysis of RDX
(Sikka *et al.*, 1978)

Minutes of Irradiation	RDX x 10^5M	(NO_2^-) x 10^5M	(NO_3^-) x 10^5M	$(NO_2^-)/(RDX)$
0	12.0	0.093	2.1	
90	11.0	2.3	3.6	0.45
135	10.0	3.5	5.8	0.59
195	8.3	4.3	5.8	0.88
225	7.2	4.6	5.5	1.1
275	6.6	5.3	5.4	1.0

Table II-6. Percentile Absorption of Light of Different Wavelengths by One Meter of Lake Water, Settled of Particulate Matter, of Several Wisconsin Lakes of Progressively Greater Concentrations of Organic Color (Wetzel, 1975)

Wavelength (mm)	Distilled Water	Crystal Lake	Lake Mendota	Alelaide Lake	Mary Lake	Helmet Lake
800	88.9	89.9	90.5	92.4	91.7	93.2
780	90.2	91.3	91.9	93.5	93.0	94.5
760	91.4	93.5	92.6	94.5	94.8	96.0
740	88.5	89.3	91.5	92.7	93.0	96.2
720	64.5	67.6	71.0	78.0	78.0	86.9
700	45.0	50.4	49.7	66.3	70.7	82.5
685	38.0	45.2	42.2	65.7	71.7	86.6
668	33.0	40.3	36.8	65.0	73.3	88.0
648	28.0	37.0	31.9	64.5	75.2	91.2
630	25.0	34.4	28.9	65.8	77.8	94.0
612.5	22.4	32.1	26.3	66.8	80.3	96.0
597	17.8	27.5	22.5	67.0	83.2	97.6
584	9.8	22.0	17.6	67.1	85.7	98.2
568.5	6.0	19.3	14.0	67.6	88.5	98.6
546	4.0	19.2	13.5	70.9	91.6	99.3
525	3.0	19.8	14.1	74.5	94.8	*
504	1.1	20.7	15.2	81.0	97.4	*
473	1.5	21.7	21.7	88.6	99.4	*
448	1.7	23.8	27.8	92.2	*	*
435.9	1.7	24.4	31.0	95.2	*	*
407.8	2.1	28.1	44.3	99.0	*	*
365	3.6	40.0	80.0	*	*	*
Color Scale (Pt units)	0	0	6	28	101	264

*not measured

Table II-7 Hydrolysis of RDX at pH 9.07 at 31°C (Sikka *et al.*, 1978)

Days	% Hyrdolysis[a]	$(NO_2^-) \times 10^6 M$	$(NO_3^-) \times 10^5 M$
0	0	3.3	-
3	4.1	4.0	5.2
5	6.4	4.7	4.5
7	9.2	5.6	-
11	16.7	9.2	-
14	22.5	10.5	2.7
18	25.4	11.9	-
21	27.0	13.0	3.8

[a] Based on RDX.

3. Biodegradation and Bioconcentration

a. Degradation by Microorganisms

No data are available in the literature on the biodegradation of SEX. However, the replacement of a nitro group by an acetyl group should render the molecule more susceptible to degradation by microorganisms. Osmon and Klausmeier (1973) tried to isolate organisms capable of using RDX as the sole source of carbon. They found organisms capable of growing on a complex media saturated with RDX, but no breakdown of RDX was observed.

Kaplan (1979) observed disappearance of ^{14}C labelled RDX from anaerobically incubated cultures inoculated with activated sludge. 1,3,5-trinitroso-hexahydro-1,3,5-triazine was identified as the reduction product. No disappearance of ^{14}C-RDX was found in aerobically incubated cultures. Sikka *et al.* (1978) studied the microbial degradation of RDX. Water collected near an RDX waste outfall (Holston AAP lines 1-5) was spiked with RDX or with RDX and sediment. Results indicate an ∿ 20 day lag time before degradation initiated. Very little degradation was observed in samples without sediment. In samples containing sediment, nearly 80% of the RDX had disappeared within 2 weeks after degradation commenced (Sikka *et al.*, 1978).

b. Bioconcentration

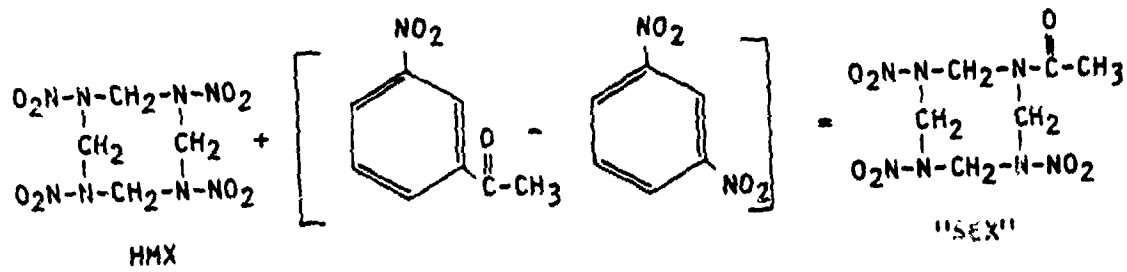
No information is available in the literature on the bioconcentration of SEX. In order to determine if bioconcentration is important for SEX, the octanol-water partition coefficient (P) and bioconcentration factor (BCF) were calculated based on experimental data for HMX. The procedure used to calculate P for SEX was that outlined by Leo *et al.* (1971). Calculational procedures and results are shown in Table II-8. A preliminary value for the partition coefficient of 1.36 for HMX was obtained at Atlantic Research (1979). The experimental procedure specified in the Federal Register (1979b) was used. The solutions were maintained at 25°C. HMX analysis was accomplished by high pressure liquid chromatography using a C_{18} μ-Bondapak column and a 30% methanol 70% water carrier phase.

As can be observed from Table II-8, the results calculated using data for dinitrobenzene and mononitrobenzene differ. Thus the addition of a nitro group and change in the symmetry of the molecule make a considerable difference in the estimated P. In addition to these effects the substitution on a benzene ring is significantly different from substitution on a 8-membered cyclic ring. Thus, the actual P measured experimentally may be significantly different from the estimated value.

Using the equation developed by EPA (Federal Register, 1979a), estimates of the bioconcentration factor for HMX and SEX in aquatic organisms are presented in Table II-9. The data indicates that neither HMX or SEX present an accumulation threat in aquatic organisms.

Table II-8. Calculation of log P for SEX

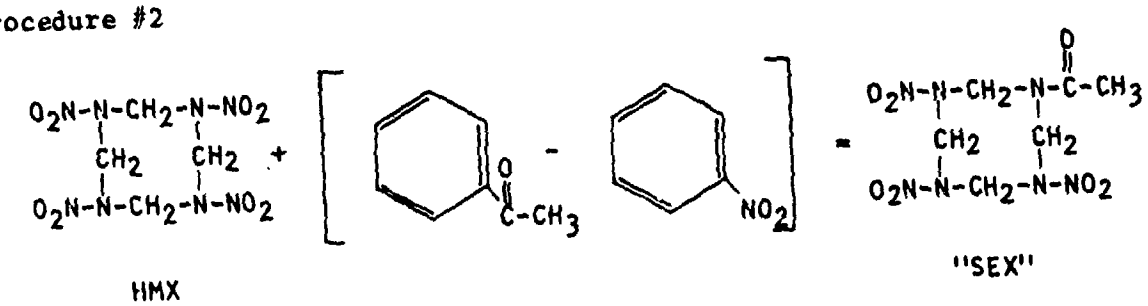
Procedure #1



HMX "SEX"

log P of HMX*	+	log P of bracketed chemicals**	=	Estimated log P of SEX	Estimated P of SEX
0.13	+	[1.42 - 1.49]	=	0.06	1.15

Procedure #2



HMX "SEX"

log P of HMX*	+	log P of bracketed chemicals**	=	Estimated log P of SEX	Estimated P of SEX
0.13	+	[1.58 - 1.85]	=	-0.14	0.72

*Atlantic Research Corporation (1979)
**log P values from Fujita *et al.* (1964); techniques from Leo *et al.* (1971)

Table II-9. Bioconcentration Factors (BCF) and Octanol-Water Partition Coefficients (P) for RDX and TAX

HMX		Estimated SEX Range	
P*	BCF**	P	BCF**
1.36	.74	.72 - 1.15	.46 - .65

*Atlantic Research Corporation (1979)
**Calculated from log BCF = 0.76 log P - 0.23 (Federal Register, 1979a)

4. Effects on Animals

a. Mammals

The only information on the effects of SEX on mammals is that inferred from known effects of HMX or RDX. These effects were discussed in Section D.3.

b. Birds

No information is available in the literature on the effects of SEX or HMX on birds.

c. Fish

No information is available on the toxicity of SEX to fish. Bentley *et al.* (1977) conducted static acute toxicity tests of nominal HMX concentrations for several species of fish. The HMX was delivered to the fish from an acetone stock solution. Barkley (1977) found the solubility of HMX in water at 20°C to be 6.6 ppm and a water - 2% acetone mixture at 20°C increased the solubility to 15.0 ppm. Barkley's data on the solubility of HMX would indicate that the maximum concentrations of HMX in the test solutions of Bentely *et al.* (1977) would be near 15.0 ppm.

Bentley *et al.* (1977) reported 96-hour LC50's for the rainbow trout (*Salmo gairdneri*), channel catfish (*Ictalurus punctatus*), bluegill sunfish (*Lepomis macrochirus*), and the fathead minnow (*Pimephales promelas*) to be greater than 32 ppm (greater than the solubility of HMX). They also tested the toxicity of HMX to fathead minnow life stages (Table II-10). A 96-hour LC50 of 15 ppm for the 7-days post hatch stage was reported. A better approximation of the actual LC50 to the 7-days post hatch stage could not be calculated because the report by Bentley *et al.* (1977) did not give percent survival or concentrations tested. If one assumes that 3.2, 5.6, 10.0, 18.0 and 32.0 ppm levels of HMX were tested, then the actual LC50 for the 7-days post hatch stage would be between 10-15 ppm. Bentley *et al.* (1977) also examined the variations in temperature, pH and hardness on the toxicity of HMX to bluegill sunfish. The ranges tested were:

Temperature (°C)	15, 20, 25
pH	6.0, 7.0, 8.0
Hardness (ppm as $CaCO_3$)	35, 100, 250

Table II-10. Acute Toxicity of HMX to Selected Life Stages of Fathead Minnows (*Pimephales promelas*) as Determined During Static Bioassays (Bentley *et al.*, 1977)

Life Stage	LC50 (mg/l) 24-hour	48-hour	96-hour	Water Parameters: Dissolved Oxygen (ppm)	pH	Temperature (°C)	Hardness (ppm as $CaCO_3$)
eggs	>32	>32	>32	8.0-8.2	7.1	20±1.0	35
1-hour post hatch	>32	>32	>32	"	"	"	"
7-days post hatch	>32	25	15	"	"	"	"
30-days post hatch	>32	>32	>32	"	"	"	"
60-days post hatch	>32	>32	>32	"	"	"	"

However, the water parameter variations tested did not change the toxicity of HMX to the fish.

Sullivan *et al.* (1979) using proposed EPA water quality criteria (Federal Register, 1978) calculated the 24-hour average concentration for HMX to be 0.92 ppm. The value of 0.92 ppm appeared to be more realistic than the criteria of 0.15 ppm calculated by taking the lowest LC50 value times a .01 application factor.

d. Amphibians

No information is available on the effects of SEX or HMX on amphibians in the literature.

e. Invertebrates

Athough no toxicity data on SEX to invertebrates were available, data on the acute toxicity of HMX to aquatic invertebrates are presented in Table II-11. Values reported were all greater than the solubility of HMX. If the toxicity of SEX to aquatic invertebrates parallels HMX, it is doubtful that the invertebrates in the Holston River will be stressed due to SEX levels released by Holston AAP.

f. Microorganisms

No information is available in the literature on the toxicity of SEX to microorganisms. However, Holston AAP effluents containing RDX, HMX, and presumably SEX and TAX were not mutagenic to the *Salmonella* strains in the Ames test (Stilwell *et al.*, 1977).

5. Effects on Plants

a. Phytotoxicity

Bentley *et al.* (1977) tested the toxicity of HMX to four algae, *Microcystis aeruginosa*, *Anabeana flos-aquae*, *Selenastrum capricornutum* and *Navicula pelliculosa*. Cell density and chlorophyll *a* were used as criteria to determine the effects of HMX. HMX increased the number of cells/ml and chlorophyll *a* content over the controls. Therefore, HMX in concentrations of 32 ppm or less were beneficial to algae growth.

Sullivan *et al.* (1979) statistically reevaluated the phytotoxicity data of Bentley *et al.* (1977). Sullivan *et al.* (1979) correctly identified questionable statistical methods and could determine significant differences in algae populations at HMX concentrations as low as 10 ppm. However, Sullivan *et al.* (1979) incorrectly reported a decrease in *A. flos-aquae* growth due to HMX.

Table II-11. EC50 of HMX (ppm) to Aquatic Invertebrates During Static Bioassays (Bentley *et al.*, 1977)

Organism	Life Stage	Hours of Exposure		Water Parameters		
		24	48	pH	Temperature (°C)	Hardness (ppm as $CaCO_3$)
Water flea (*Daphnia magna*)	0-24 hours	>32	>32	7.1	20±1.0	35
Scud (*Gammarus fasciatus*)	juvenile	>32	>32	"	"	"
Sowbug (*Asellus militaris*)	"	>32	>32	"	"	"
Midge (*Chironomus tentans*)	2nd-3rd instar	>32	>32	"	"	"

b. Bioaccumulation and Degradation

No information was found on the bioaccumulation or degradation of SEX or HMX by plants.

F. Regulations and Standards

There are no regulations or standards for SEX in the United States.

G. Evaluation and Comments

The information on SEX is limited to physical and chemical data and effluent data from Holston AAP. No toxicological or environmental data on this compound were found in the literature search or through personal contacts. The toxicological and environmental properties of SEX were inferred from limited data available on the related compound HMX. HMX in dimethylsulfoxide is moderately toxic to guinea pigs with an intravenous LD50 of 28.2 mg/kg. The toxicity of HMX to aquatic organisms is low. The most sensitive organism was the 7-day post hatch fathead minnows with an LC50 of 15 ppm. The presence of the $>N-\overset{O}{\overset{\|}{C}}-CH_3$ group in SEX is expected to increase the biological activity of this compound over that of HMX. However, the extent of this increase is not predictable.

It is estimated that Holston AAP would discharge 0.4 to 0.8 million lb of SEX/year under full mobilization production scheduled. The release of these quantities of this compound should be of major concern to the Army.

The following studies are recommended in order to further assess the toxicological and environmental hazards associated with this compound:

- Laboratory studies should be conducted to further define the solubility properties and aqueous chemistry of SEX. These studies should include solubility determination of SEX in aqueous medium under different pH conditions. Reactivity and potential decomposition of SEX as a function of pH and anions should also be determined. The aqueous solubility or reactivity of SEX in the presence of small amounts of organics such as acetone, cyclohexanone and formaldehyde also requires further study.

- Further sampling and analysis of Holston AAP effluents, the Holston River and river sediment are needed to verify existing data. However, before these studies are undertaken, the extraction and analytical methodology requires further investigation. This investigation should determine the optimum methods for sample storage, extraction and analysis in order that repeatable and reliable data may be obtained. Reliable analytical standards should also be prepared.

- No toxicological data exists for SEX. Therefore, the following studies are recommended:

  1. Acute mammalian studies should be conducted including i.v. injection of a SEX/DMSO solution in mice for comparison with HMX. An oral feeding study with mice or

rats should also be undertaken. If these studies show that SEX is highly toxic, chronic toxicological studies may be warranted.

2. Acute and chronic aquatic toxicity studies should be conducted with species of fish and invertebrates found in the Holston River.

3. Bioaccumulation and biodegradation studies with SEX in aquatic organisms and microorganisms should be conducted. These studies will help determine the environmental fate of and potential hazards from this compound.

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PROBLEM DEFINITION STUDY ON
LEAD SALICYLATE AND LEAD β-RESORCYLATE

SUMMARY

Lead β-Resorcylate

Lead β-resorcylate is used as a burning rate modifier in solid propellant formulations. The Army is the main user of this chemical in the United States. Radford AAP effluents are probably the main source of pollution of this salt. The environmental fate of lead β-resorcylate has not been determined. By analogy to other lead com exes, it is probably hydrolyzed to inorganic lead and precipated as the carbonate or sulfate.

The toxicity of lead β-resorcylate to mammals is unknown. In acute doses, lead β-resorcylate is expected to show low toxicity if comparison with lead salicylate is valid. However, chronic toxic effects could be a problem.

The aquatic toxicity of lead β-resorcylate is unknown but should be less than lead acetate. This toxicity will also vary with water parameters.

Five studies are recommended in order to fill in the information gaps on lead β-resorcylate:

1. Further enumeration of the composition, chemistry, and analysis methods for salts
2. Sampling and analysis at Radford AAP to determine amounts of lead β-resorcylate in the effluent and accumulation in the New River sediment and biota
3. Acute mammalian toxicity study
4. Chronic mammalian toxicity study
5. Acute and chronic aquatic toxicity tests with fish and invertebrates.
6. Determine the effectiveness of proposed treatment facilities to remove lead β-resorcylate from Radford's AAP effluents.

Lead Salicylate

Lead salicylate is used by the Army as a burning rate modifier in solid propellant formulations. These propellants are currently only in production at Radford AAP, although Badger and Sunflower AAP's would also use lead salicylate when these plants are operational.

Most of the information on the toxicological and environmental properties of lead salicylate must be inferred from experimental data on lead acetate. From the one piece of information available on mammalian toxicity, lead salicylate is relatively non-toxic to rats when administered in an oral acute dose. No chronic toxicity data is available; however, the chronic effects of lead salicylate are expected to be similar to lead acetate.

In the aquatic environment, hydrolysis of lead salicylate is expected to occur. Sufficient data are not available to assess the toxicity of lead salicylate to aquatic life.

In order to fill the information gaps, the following studies are recommended:

- further investigation of the physical and chemical properties of lead salicylate
- chronic feeding study to determine the long-term effects of exposure to lead salicylate
- an acute skin LD50 to determine skin absorption
- aquatic toxicity studies with fish and invertebrates.

FOREWORD

A. Study Goals

This report presents the results of an evaluation of the available information on the toxicological and environmental hazards of lead salicylate and lead β-resorcylate. Lead salicylate and lead β-resorcylate are used by the Army as a burning rate moderators in solvent and solventless double base propellants. These salts enter the environment in the wastewater generated during the blending of propellant ingredients at the Army Ammunition plants. The wastewaters generated at the Army propellant manufacturing facilities are a major source of entry of lead salicylate and lead β-resorcylate into the environment. The evaluation of toxicological and environmental hazards of lead salicylate and lead β-resorcylate was undertaken in order to aid the Army in identification of research needs and in recommendation of environmental criteria for these compounds.

B. Study Methodology

The methodology utilized to gather information for this report included a detailed search of the literature and numerous personal contacts. During the literature search, the following sources were reviewed for pertinent information on lead salicylate and lead β-resorcylate.

- Chemical Abstracts	1940 - present
- Biological Abstracts	1950 - present
- Excerpta Medica	1950 - present
- TOXLINE	1965 - present
- National Technical Information Services	1964 - present
- Defense Documentation Center	1958 - present
- COMPENDEX	1970 - present

Personal contacts were made with U.S. and foreign manufacturers, Army Ammunition Plant personnel and Army and civilian researchers.

1. Contacts with U.S. Manufacturers

Mr. Don Hurley, NL Industries, Sept. 27, 1978. Mr. Hurley said that lead salicylate and lead β-resorcylate were never used in medicines or pharmaceutical preparations. Therefore, no toxicological studies were ever performed. NL Industries uses the same precautions that are applicable to other toxic lead compounds.

Mr. Ted E. Potter, Environmental Manager, The Shepherd Chemical Co., Sept. 22, 1978. Mr. Potter supplied an oral LD50 for lead salicylate of 4.3 gm/kg in the rat. No information was available on lead β-resorcylate.

2. Foreign Contacts

Nine foreign companies listed in the 1978 Directory of Chemical Producers in Western Europe were contacted by Telex in October, 1978.

FRANCE
Melle-Bexons SA
Rhone-Poulenc Industries SA

FED. REP. OF GERMANY
Akzo Chemie GmbH
Chemische Werke Munchen Otto
Barlocher CmbH
Metallgesellschaft AG

ITALY
Stabilital SpA

SPAIN
Industrias Quimicas de Parets. SA

UNITED KINGDOM
Akzo Chemie UK Ltd.
Hopkin & Williams

Four companies responded, none of them had any toxicological information.

3. RAAP Personnel

The following RAAP personnel were contacted Sept. 28, 1978:

Mr. John Horvath
Mr. Tom Grady
Mr. Ted Topper

Mr. Horvath had no information. Mr. Grady suggested calling Dr. Emil Christifano of Hercules Inc. or NL Industries since they produce the compound. Mr. Topper searched for toxicological information but found none.

4. Other Sources

Dr. Jay Abercrombie of the U.S. Army Chemical Systems Laboratory, Aberdeen Proving Ground, Md., was contacted Sept. 12, 1978. He reported no information of the lead compounds.

Mr. J. Gareth Pearson of USAMBRDL, Fort Detrick, Md., was visited Sept. 1978. Data on the aquatic toxicities of lead salicylate and lead β-resorcylate were provided.

Dr. Emil Christifano and Mr. Tom Butler of Hercules, Inc., were contacted on October 2, 1978, for toxicological information on lead salicylate and lead β-resorcylate. They had no specific data.

The Department of Transportation was contacted in October 1978, for any toxicological information on lead salicylate and lead β-resorcylate. However, it had no specific information.

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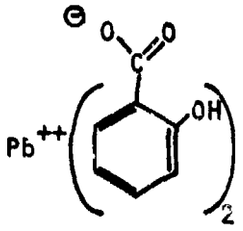
III. Lead Resorcylate and Lead β-Resorcylate

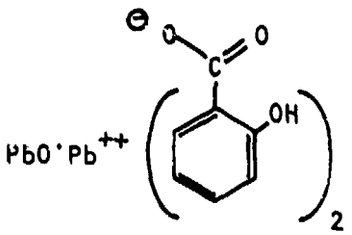
A. Alternate Names

Lead salicylate and lead β-resorcylate are divalent lead salts of salicylic acid and β-resorcylic acid. These salts are produced by the reaction of lead oxide and the corresponding acid.

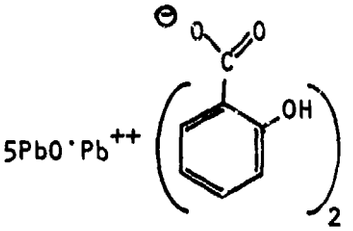
Lead salicylate is produced by NL Industries. This salt has been isolated in three forms, namely the normal lead salicylate which has a molecular weight of 481 and the mono and pentabasic varieties which have molecular weights of 704 and 1596 respectively. Structural formulae of the three forms are shown below:



"Normal"



Monobasic

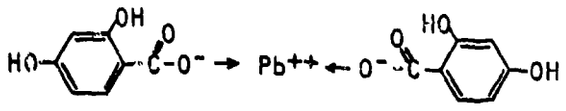


Pentabasic

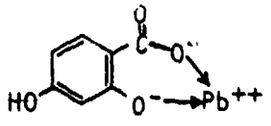
Pertinent alternate names for lead salicylate are listed below:

CAS Registry No.:	15748-73-9
Alternate Registry No.(s):	16183-13-4; 14901-86-5; 824-37-3
C.A. Name (9CI):	Lead, bis(2-hydroxybenzoate-O^1, O^2), (T-4)-
C.A. Name (8CI):	Lead, bis(salicylato)-
Synonyms:	Salicylic acid, lead(2+)salt(2:1); Benzoic acid, 2-hydroxy-, lead(2+) salt(2:1)

Lead β-resorcylate is obtained commercially from NL Industries and Shepherd Chemical Company. Commerical lead β-resorcylate is apparently a mixture of dibasic lead β-resorcylate, monobasic lead β-resorcylate and possibly a third variety.



dibasic lead β-resorcylate



monobasic lead β-resorcylate

Analysis of this third salt indicates that it has a 7:4 lead to resorcylate ratio (Satriana, 1971a).

The pertinent alternate names for lead β-resorcylate are listed below:

Mixture

CAS Registry No.:	20936-32-7
CA Name (9CI):	Benzoic acid, 2,4-dihydroxy, lead salt
CA Name (8CI):	beta-resorcylic acid, lead salt
Synonyms:	Lead beta-resorcylate; Lead 2,4-dihydroxybenzoate

Monobasic

CAS Registry No.:	41453-51-4
Molecular Formula:	$C_7H_4O_4Pb$
CA Name (9CI):	Benzoic acid, 2,4-dihydroxy, lead (2+) salt(1:1)
Synonyms:	2,4-dihydroxybenzoatolead(II)

Dibasic

CAS Registry No.:	41453-50-3
Molecular Formula:	$C_7H_5O_4 \cdot 1/2\ Pb$
CA Name (9CI):	Benzoic acid, 2,4-dihydroxy, lead(2+) salt(2:1)
Synonyms:	Bis(2,4-dihydroxybenzoate)lead(II0

B. Physical Properties

The available physical properties of lead salicylate and lead β-resorcylate are listed in Tables III-1 and III-2. Satriana (1971a) reported solubility data, infrared spectra, x-ray diffraction patterns, thermograms and thermogravimetric data for monobasic and dibasic lead β-resorcylate and the third variety (designated sample 62-1 by Satriana). His data are presented in Figures III-1 through III-4 and Table III-2. The infrared spectra and x-ray diffraction patterns shown in Figures III-1 and III-2 indicate that the three salts are different in composition and structure.

C. Chemical Properties

1. Lead Salicylate

a. General Chemistry

Very little literature could be found pertaining to the chemistry of lead salicylate. The synthesis of lead salicylate can be accomplished by a number of methods which relate to its acid/base reactivity. By adding salicylic acid to a basic solution of lead acetate or lead chlorid

Table III-1. Physical Properties of Lead Salicylate*

Physical Form @ 20°C:	soft crystalline powder
Color:	creamy white
Crystal structure:	planar
Melting Point:	no data available
Volatility:	no data available
Vapor Pressure:	no data available
Specific Gravity:	2.3 normal; 3.32 monobasic; 5.11 pentabasic
Refractive Index:	1.78, normal; 1.90 monobasic; 2.05 pentabasic
Solubility	soluble in hot water, alcohol (no specific numbers available)
Magnetic susceptibility:	-0.4185×10^{-6} (monohydrate)

*Hawley, 1977; Nat. Lead Co., 1947; Kebrich, 1947; Prasad *et al.*, 1947.

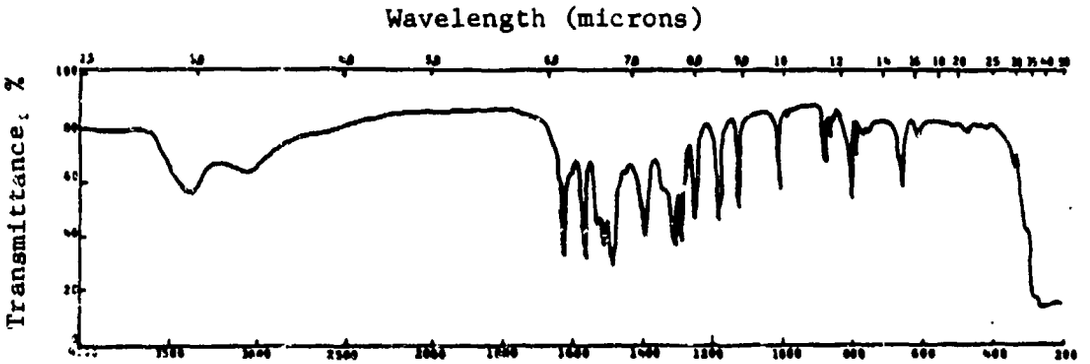
Table III-2. Physical Properties of Lead β-Resorcylate*

Physical Form @ 20°C:	powder
Color:	creamy white
Melting Point:	no data available
Volatility:	no data available
Vapor Pressure:	no data available
Octanol-water partion coefficient	no data available

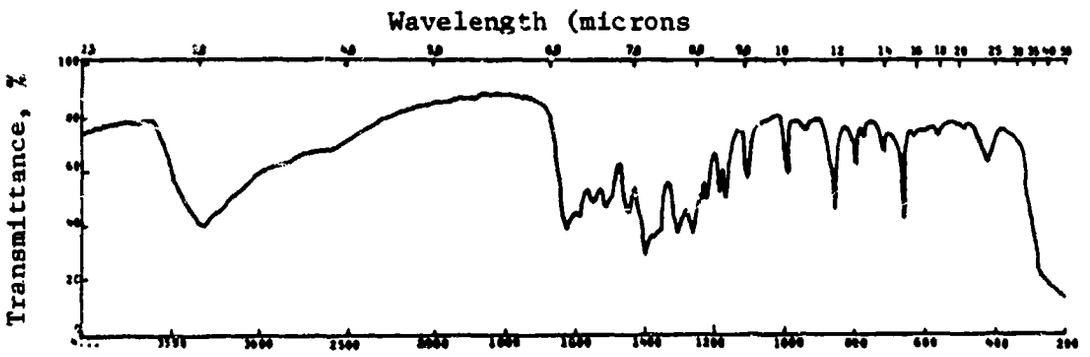
Solubility:
(no specicific numbers available)

Solvent	Dibasic Salt	Monobasic Salt	Sample 62-1
water	sl. sol.	insol.	insol.
95% ethanol	sol.	insol.	insol.
dimethylformamide	sol.	insol.	insol.
acetone	sol.	insol.	insol.
tetrahydrofuran	sol.	insol.	insol.
dimethylsulfoxide	sol.	insol.	insol.
benzene	sl. sol.	insol.	insol.

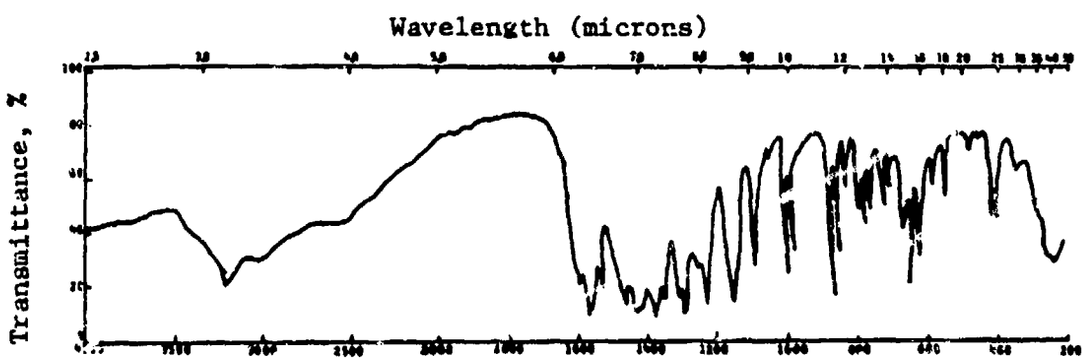
*Satriana, 1971a



Dibasic Lead β-Resorcylate



Monobasic Lead β-Resorcylate



Unidentified Lead β-Resorcylate Salt (Sample 62-1)

Figure III-1. IR Spectra of Three Lead β-Resorcylate Salts (Satriana, 1971a)

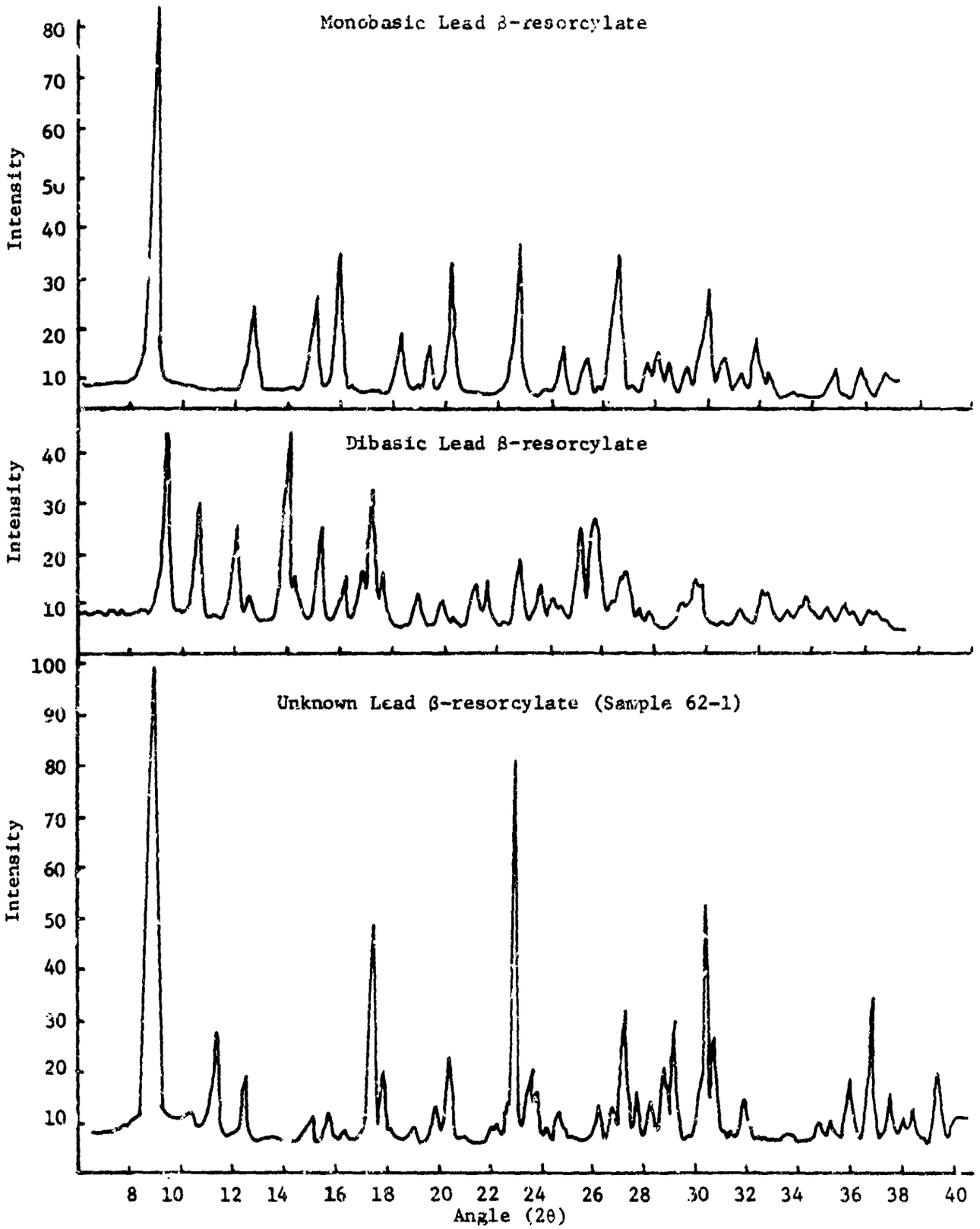
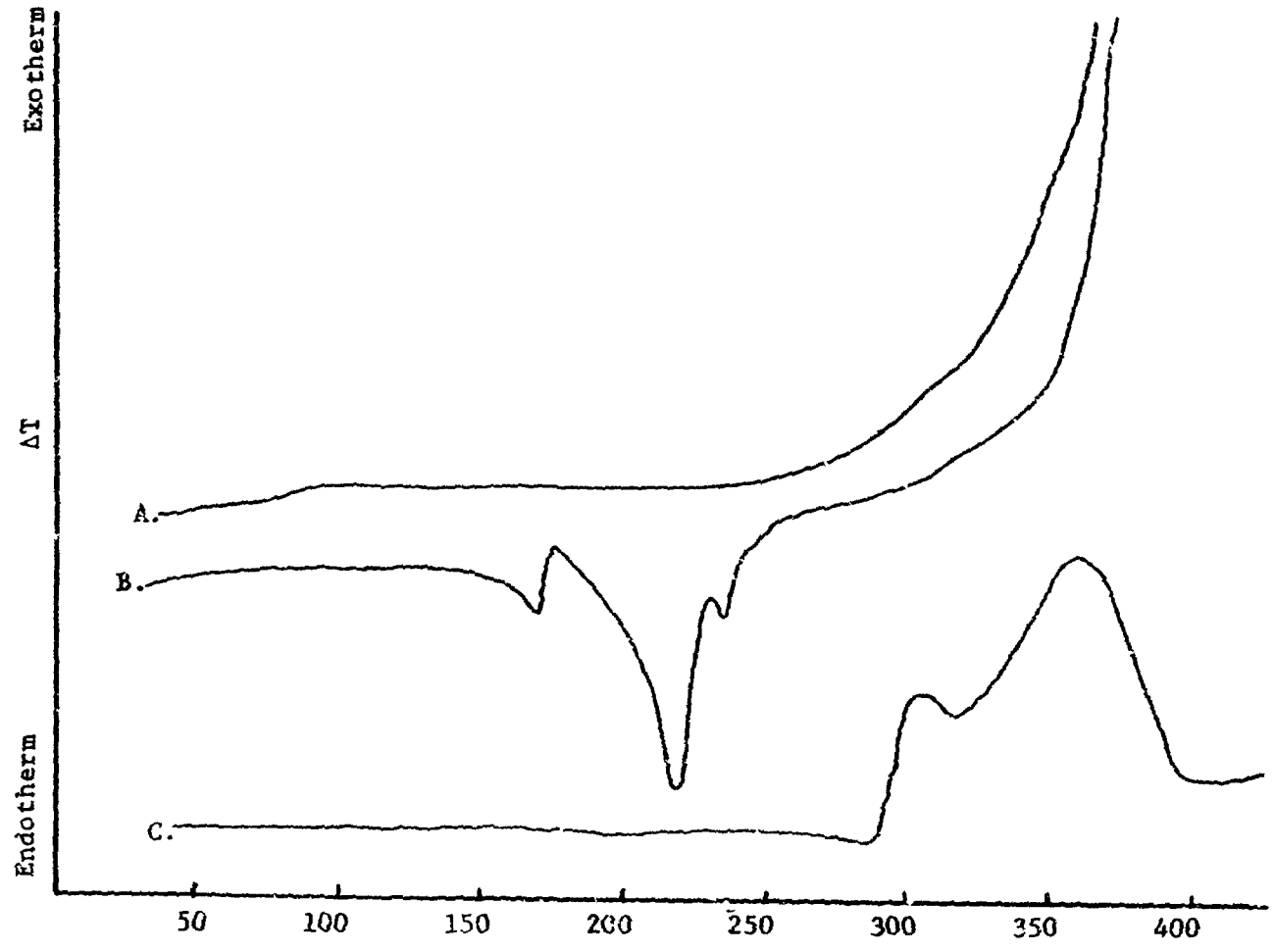
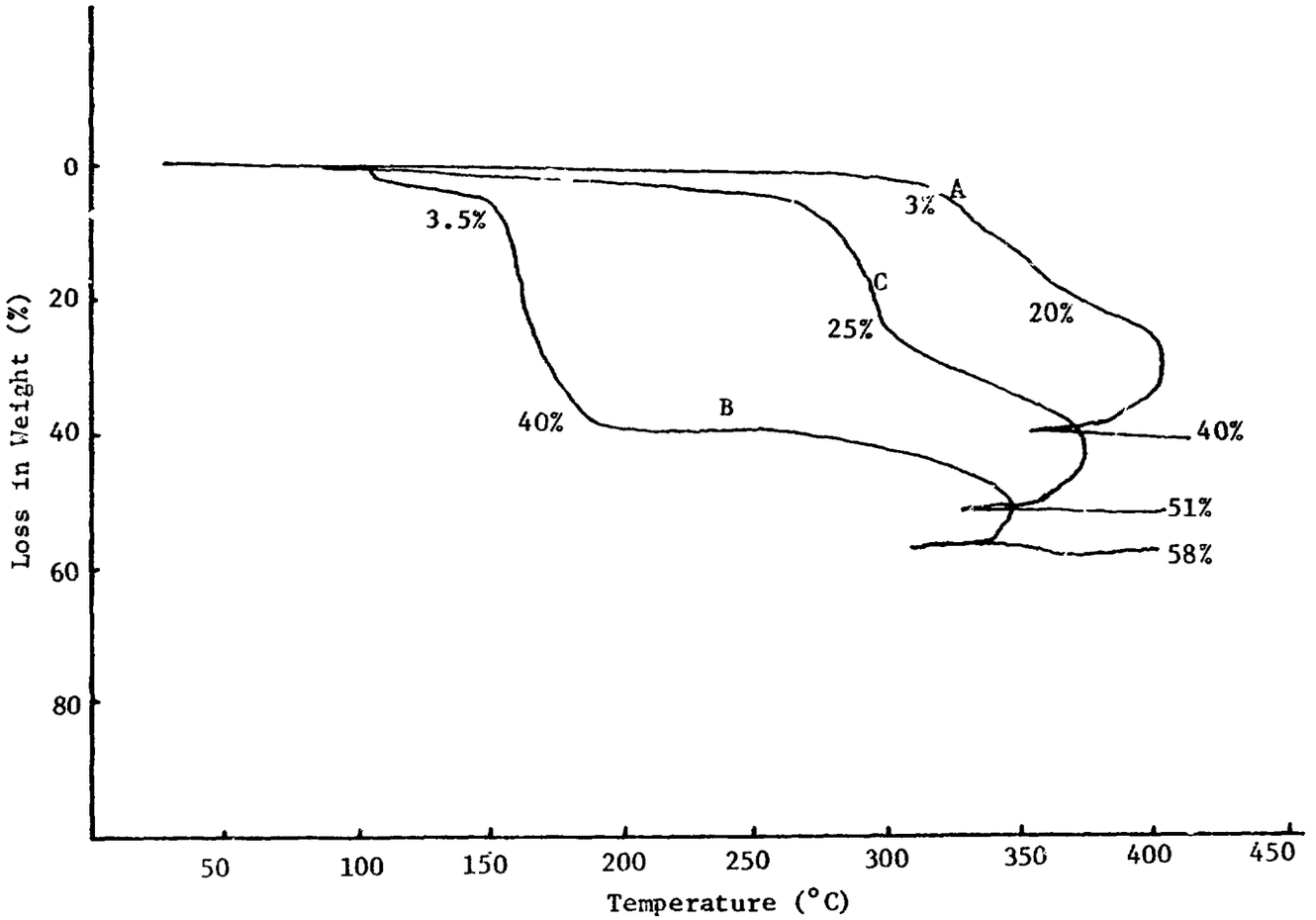


Figure III-2. X-ray Diffraction Patterns of Three Lead β-Resorcylate Salts (Satriana, 1971a)



A. Monobasic lead β-resorcylate
B. Dibasic lead β-resorcylate
C. Sample 62-1

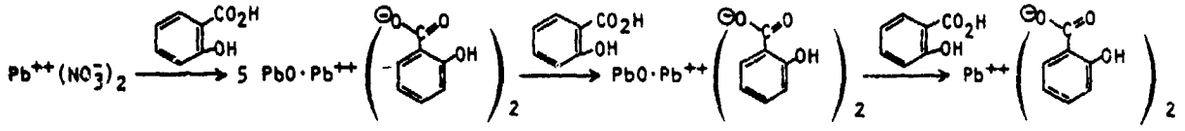
Figure III-3. Thermograms of Three Lead β-Resorcylate Salts. (Satriana, 1971a; Satriana, 1971b)



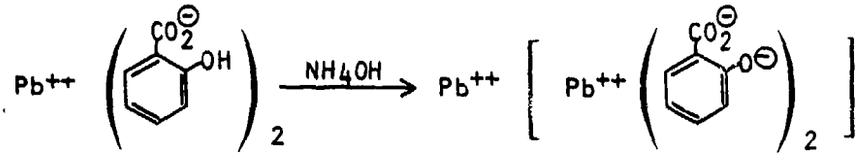
A. Monobasic lead β-resorcylate
B. Dibasic lead β-resorcylate
C. Sample 62-1

Figure III-4. Thermogravimetric Trace of Three Lead β-Resorcylate Salts. (Satriana, 1971a; Satriana, 1971b)

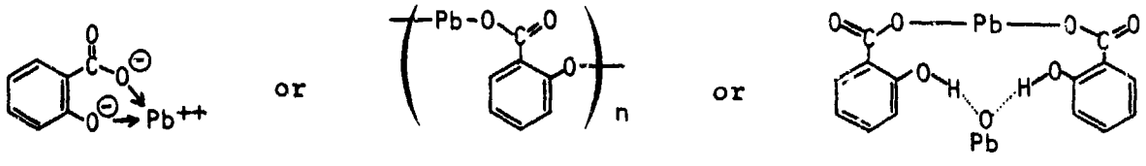
the formation of pentabasic lead salicylate at pH 9.9 is observed. At pH 8.3, the monobasic variety is formed and finally at pH 4.8, the normal salt is obtained (Kebrich, 1947; Kebrich, 1946; Nat. Lead Co., 1947).



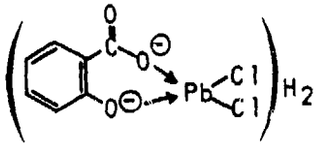
The monobasic salt has also been prepared by raising the pH of a solution of the normal salt (Murgulescu and Dobrescu, 1948).



Several different molecular formulas are presented in the literature for the monobasic lead salicylate salt. These formulas differ only by a molecule of water. The actual structure could be presented as a dianionic salicylate of a 1:1 adduct of lead oxide and the normal salt:

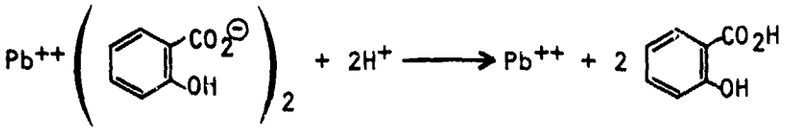


There are widely divergent reports as to the stability of lead salicylate in aqueous media. Formation constants of 10 and 83, respectively, have been reported for Pb(salicylate)+ and Pb(salicylate)$_2$ from polarography data (Jain and Gaur, 1966). Other authors concluded from a similar polarographic study that lead does not form a stable complex with salicylate ion (Vinogradova and Pedanova, 1956). In acidic solutions, protonation of the salicylate ions should lead to rapid hydrolysis of the salt; however, the synthesis of an acidic molecule comparable in strength to H_2SO_4 has been reported (Lesbre, 1938). This species has been assigned the following structure:

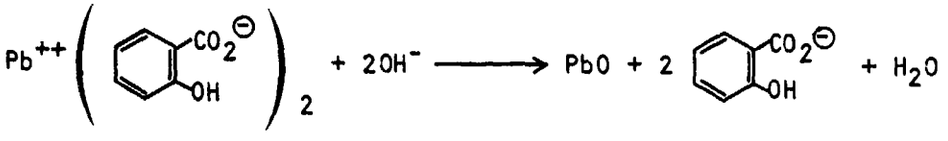


b. Environmental Reactions

The most probable environmental reaction of lead salicylate would be hydrolysis, ideally in acidic solution, to lead ion and salicylic acid:



Hydrolysis could also occur in basic solution although more slowly:



2. Lead β-Resorcylate

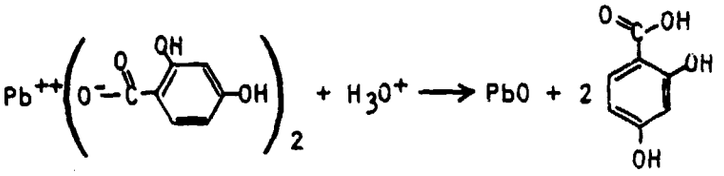
a. General Chemistry

The only literature references to the chemistry of lead β-resorcylate are those of Satriana (1971a; 1971b) who investigated the synthesis of the lead β-resorcylates and the adsorption of water by dibasic lead β-resorcylate. Pure monobasic lead β-resorcylate was obtained in good yield when lead oxide and resorcylic acid were mixed in a 1:2 ratio in an alcoholic solution. Good yields of the pure dibasic salt were obtained when the resorcylate concentration was increased to three times that of the lead oxide. When water was the solvent in the synthesis, an unknown salt resulted.

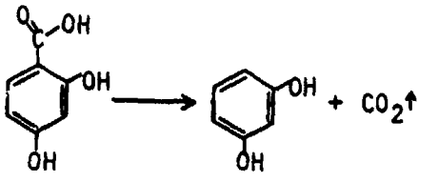
Differential thermal analysis (DTA) and thermogravimetric analysis (TGA) curves of lead β-resorcylate are shown in Figures III-3 and III-4. The DTA and TGA curves of the dibasic salt suggest the reversible loss of a water molecule at approximately 100-150°C. All three salts showed larger weight losses consistent with loss of the resorcylate anions at higher temperatures. Propellants containing lead β-resorcylate have been shown to ooze resorcylic acid on aging. This loss is due either to hydrolysis or expulsion of the anhydride (Satriana, 1971b).

b. Environmental Reactions

No data on the environmental fate of lead β-resorcylate was found in the literature search. One would expect the initial formation of lead oxide and resorcylic acid via direct hydrolysis.



The resorcylic acid would in all probability decarboxylate yielding resorcinol.



D. Monitoring and Analysis

1. Analytical Methods

Quantitative analysis for lead salicylate and lead β-resorcylate may be accomplished by oxidation to lead oxide. The lead can then be determined by atomic absorption. Conversion to lead dithizone allows quantitative determination of the lead content by absorption at 520 nm (Franson, 1975). This method can be used to determine 0-75 µg Pb in the presence of 100 µg of Sb, Sn, Hg, Ag, Cu, Cd, As, Fe, Al, Ni, Co, Mn, Zn, Sr, Ca, Mg, Na, K, NH_4. Thin layer chromatography has been employed for analysis of lead β-resorcylate in solid propellant mixtures containing hexanoic, stearic and salicylic acid salts of copper and lead (Habermann, 1971). No detection limits were given. Quantitative determination of the salicylate and resorcylate in propellants has also been accomplished by gas chromatography. The propellant mixtures were first treated with a 1:1 mixture of trimethylsilylchloride and bis-(trimethylsilyl) acetamide to convert the salicylate or resorcylate to its trimethylsilyl ester. The products were then chromatographed at 70-190°C on a 3% UCW-98/Gas-Chrom Q column (Alley and Dykes, 1973). Smallest quantities reported analyzed were 3.11 mg for salicylic acid and 3.15 for β-resorcylic acid corresponding to about 1.5 µg in the 1 µl injections. Mean errors were 0.01 mg for salicylate and 0.00 for the resorcylate. No lower detection limit was given.

2. Monitoring

No reference to existing environmental or industrial monitoring data on lead salicylate or lead β-resorcylate was found in the literature.

E. Health Effects

1. Biology

Studies on the biological interactions of the lead β-resorcylates and the lead salicylates have not been reported in the literature. Therefore, the effects of these compounds on and their interactions with the mammalian body must be inferred from studies on other lead compounds. Lead acetate is the only lead compound similar to lead salicylate or lead β-resorcylate for which toxicological studies have been performed. Therefore, much of the data presented herein is for lead acetate and the effects of lead salicylate and lead β-resorcylate have been inferred from these data.

a. Absorption

Several studies have been conducted in order to determine the factors that influence lead absorption through the gastrointestinal tract in man. Under normal conditions, only 8-12% of the lead ingested by man is absorbed into the body. The main absorption site is the small intestines. The colon also absorbs some lead but none is absorbed in the stomach (Harvey, 1970). The main factor controlling lead absorption appears to be the motor activity of the bowel. In his studies with human subjects, Kehoe (1942) found no effect of dietary changes in calcium or phosphorus on lead absorption. Barltrop and Meek (1975) observed no definite differences between the absorption of inorganic and organic lead compounds in the gastrointestinal tract of rats. However, they did find that an increase in the dietary fat increased the amount of lead absorbed. The lead content, from lead acetate, of rat kidney was increased from 11.4 to 20 mg when 7.5% corn oil was added to the diet.

In contrast to the low percentage of ingested lead compounds absorbed through the gastrointestinal tract, absorption of lead and lead compounds by the respiratory tract is more rapid and more complete. Estimates of percentage of lead absorbed by the respiratory tract are 37% (Beliles, 1975). Absorption occurs in all portions of the respiratory tract and is dependent on three processes: deposition, mucociliary clearance and alveolar clearance. The deposition of lead containing particles in the respiratory tract is controlled mainly by the size of the particles, inhalation route, respiration rate and tidal volume. Large particles are generally trapped in the nasopharyngeal system. For smaller particles, deposition in the tracheobronchial system and lungs occurs. Once deposition has taken place, lead particles can be removed from the respiratory system by mucociliary clearance anad alveolar clearance (Task Group on Metal Accumulation, 1973). Mucociliary clearance occurs as a result of mucous flow and ciliary activity in the nasopharyngeal and tracheobronchial systems. This clearance results in expectoration of the particles on translocation to the gastrointestinal tract. Alveolar clearance is the result of three processes (Task Group on Metal Accumulation, 1973).

- The particles can be transported from the alveoli to a region where mucociliary action will remove the material from the respiratory tract.

- The lead particles can pass through the membranes into pulmonary tissues.

- The lead particles can pass through the pulmonary tissue into the blood or lymph system.

No absorption of inorganic lead occurs through the skin. However, organolead compounds such as tetraethyllead are known to rapidly penetrate the skin. Rostogi and Clausen (1976) showed that lead acetate and lead naphthenate were absorbed into the body of rats when a solution of this compound was coated on their skins. Based on the absorption data for other lead compounds, it is expected that lead salicylate and lead β-resorcylate can be absorbed into the body via the respiratory and gastrointestinal tracts and through the skin. The respiratory tract will probably be the main absorption site, especially in occupational exposures. However, skin absorption may also be significant.

b. Transport

After absorption, lead is initially distributed to the soft tissues. While in the blood, inorganic lead is associated with the erythrocytes. The kidneys and liver are the main target organs for initial lead deposition. The biological half-life of lead in tissues is estimated at a few weeks (Clarkson, 1978).

Studies by Baxter *et al.* (1977) were carried out on 85-day old B6CF$_1$ Argonne bred female mice to determine differences in early retention of lead acetate and lead citrate at intervals up to 14 days. Tissues examined were liver, spleen, kidney, femur, lung, brain and blood. Ultrafilterable lead acetate or lead citrate containing labeled lead was intravenously injected at a level of 1 mg/kg (pH 5.1). After 1 hour the distributions of lead acetate and lead citrate were as follows (reported as percentage injected dose per g of tissue, % ID/g):

Organ	Lead Acetate % ID/g	Lead Citrate % ID/g
liver	51.1	17.6
spleen	18.5	3.6
kidney	35.3	63.4
femur	11.3	22.5

In animals given lead acetate, there was a tendency to accumulate more lead in the lungs and less in the brain and blood in one hour than in animals given lead citrate. During days 1-14, animals given lead acetate showed a greater loss of lead from the liver and spleen, whereas animals given lead citrate showed a greater loss from the kidneys. Evidence from this study suggests that lead acetate undergoes hydrolysis in blood and is more rapidly converted to the inorganic lead than is lead administered as the citrate, which is a more stable complex.

Lead can also penetrate the placental and the blood-brain barrier. Organo lead compounds such as tetraethyllead penetrate this barrier more rapidly than inorganic lead compounds. This penetration is often by a partially metabolized compound (Task Group on Metal Accumulation, 1973). Penetration of the blood-brain barrier by organic lead salts such as lead β-resorcylate and lead salicylate have not been studied.

Lead compounds initially deposited in the soft tissues are gradually redistributed and the lead deposited in the bones, teeth and hair (Harvey, 1970). Deposition of lead in the bones resembles calcium deposition. High phosphate intake is necessary for bone deposition of lead, as the lead is deposited in the form of tertiary lead phosphate (Harvey, 1970). Vitamin D aides in lead deposition in the bones. However, bone lead content is highly dynamic. Low phosphate and/or high calcium intake, acidosis, and the presence of iodides and bicarbonate favor removal of lead from the bones. The biological half-life of lead in bone is ∿10 years unless body conditions favor its removal (Clarkson, 1978).

c. Metabolism

In the body, organo lead compounds can be slowly biotransformed. If this transformation is carried to completion, inorganic lead will result. In studies by Baxter *et al.* (1977), evidence of *in vivo* hydrolysis of lead acetate and lead citrate was observed. The rate and degrees of hydrolysis are dependent on the stability of the lead complex. Thus, *in vivo* hydrolysis of lead salicylate and lead β-resorcylate can be expected to occur slowly.

d. Elimination

Lead is eliminated from the body through the feces and urine. The fecal lead content is mainly that lead which was not absorbed by the body, although there is some evidence that absorbed lead is excreted in the bile. Klaassen and Shoeman (1974) conducted a study of the biliary excretion of lead and concluded that the liver may have an active transport mechanism for lead excretion. However, Kehoe (1961) found that inhaled lead did not reach the feces of human subjects and the major portion of absorbed lead is excreted in the urine This excretion route is favored by those mechanisms which tend to mobilize lead from bone and soft tissue and by the presence of chelating agents such as dimercaprol, calcium disodium edetate (ethylenediamine tetraacetic acid) and penicillamine.

e. Pharmacology

Lead preferentially binds sulfur and sulfur containing organics forming compounds and complexes of greater stability with this element than with oxygen or nitrogen containing organics. Thus, lead is expected to exert a major influence on SH-dependent enzymes by combining with the sulhydryl group. Lead also binds with oxygen and nitrogen functional groups of proteins, enzymes, etc. Literature evidences suggests that administration of lead complexes such as lead acetate results in these types of interferences.

Lead interferes with *in vivo* biosynthesis of heme, globin synthesis in the erythrocytes and with the utilization of iron. A schematic showing the various points of lead interference is presented in Figure III-5. Lead inhibits the synthesis of prophobilinogen (Step #2) by interfering with the SH-containing enzyme, δ-aminolevulinic acid dehydratase (ALAD). The interference with heme synthetase (Step #6) is based on experimental evidence. Other interference mechanisms have not been clearly established.

Other effects of biochemical interactions of lead have been documented. For example, Ono *et al.* (1973) showed that intraperitoneal injection in mice of 0.3 ml of a 0.9% saline solution containing 1 mM lead acetate caused an increase in SH groups in the kidney over that in controls. Thus, the amount of SH in mouse kidney may be an indication of the degree of disturbance caused by lead or other heavy metals.

Hrdina *et al.* (1976) examined the effect of heavy metals on brain biogenic amines in the rat. In part of the study, rats were chronically fed lead acetate at levels of 0.2 and 1.0 mg/kg/day for 45 days. This treatment caused an increase in cerebrocortical acetylcholine and a decrease in brain stem norepinephrine. The changes in the regional levels of these biogenic amines occur before outward toxic effects become manifest. Thus, these changes may be early signs of adverse effects of lead on the central nervous system.

Cornell and Filkins (1974) administered 5 mg lead acetate intraveneously to male rats. Gluconeogenesis was determined *in vivo* by measuring conversion of labeled alanine into glucose and *in vivo* by using isolated hepatocytes. Results showed that lead treated rats sustained a depression in the conversion of labeled alanine into blood glucose. In isolated hepatocytes, gluconeogenesis from alanine, lactate or pyruvate was reduced 40-60%. It was suggested that there is a locus of lead action in the mitochondria and that defects in the regulation of glucose by the liver may exert some effect in the toxicity of acute lead poisoning.

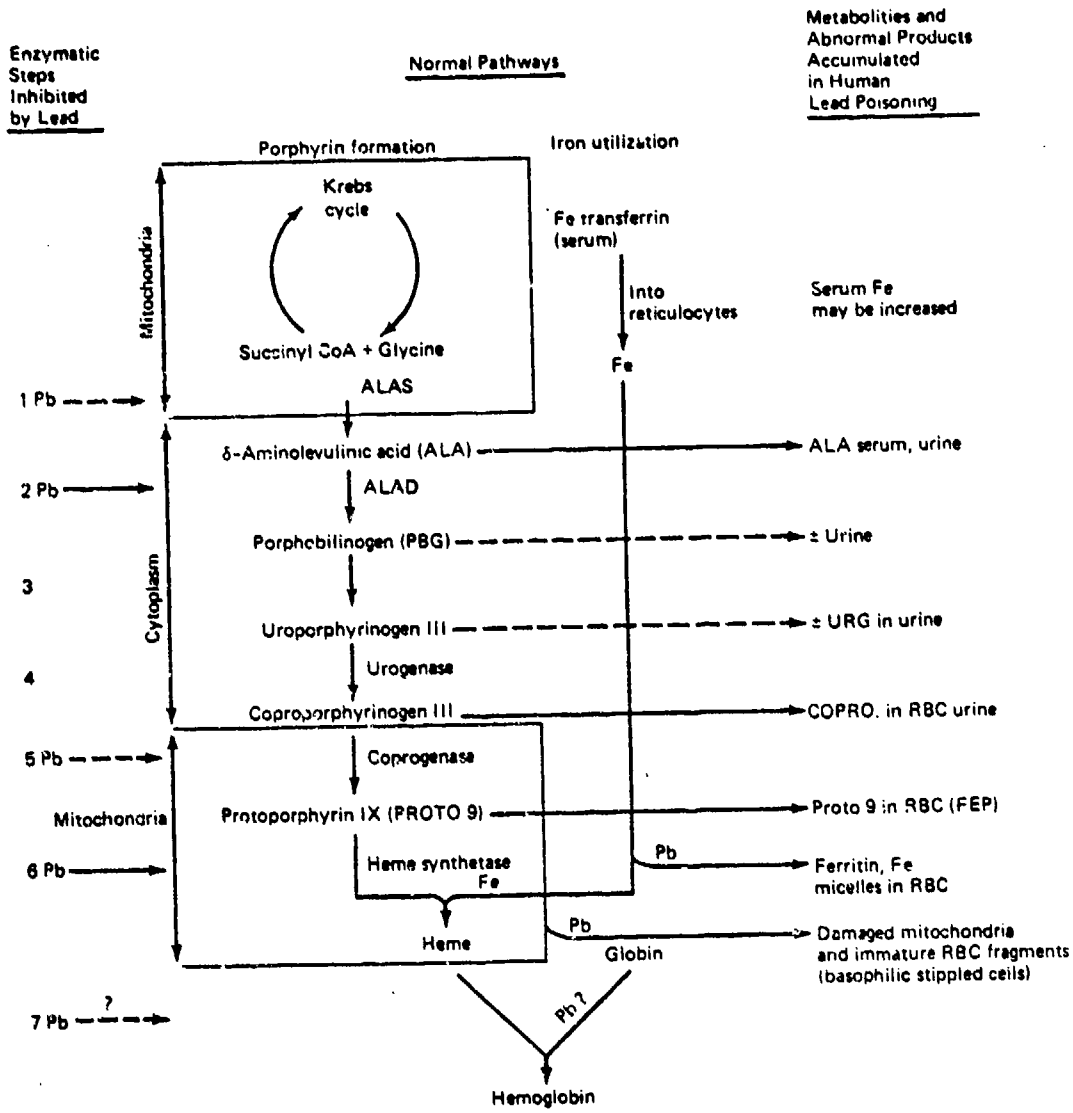


Figure III-5. Biosynthesis of Heme Showing Points of Lead Interference (Beliles, 1975)

Studies on the effect of experimental lead poisoning on the permeability of the lysomal membrane of the rat were carried out by Apostolov *et al.* (1977). Results showed that as early as the third day of a daily feeding of 20 mg of lead acetate/kg body weight, two lysomal enzymes, alpha-mannosidase and beta-acetylglucosaminidase, were found to have been activated in the blood serum. Thus, in the pathogenesis of lead poisoning, damage to the lysosomal membrane may play an important role.

2. Effects of Human Exposure

a. Epidemiology

No information was found on the epidemiology of lead salicylate or lead β-salicylate. However, chronic lead poisoning in man is well documented. The effects of lead poisoning observed in man are listed in Table III-3.

b. Occupational Exposure Studies

Occupational exposure studies on lead salicylate and lead β-salicylate have not been conducted. However, several studies with lead acetate have been performed. Cytogenetic studies were carried out on 11 male volunteers (20-30 years) who ingested lead acetate for 49 days. The concentration of lead in the blood was maintained at 400 ppb from the third week. At the end of the study, the lymphocytes showed increased mitotic activity but no increase in chromosome abberations (Bijlsma and De France, 1976).

In a review of the effects of lead on the female and reproduction, Rom (1976) points out that there is biologic evidence that women may be more susceptable to the toxic effects of lead. In animal studies, lead exerts a markedly deleterious effect on pregnancy and fetal development. A still unresolved question is whether lead is responsible for chromosomal abberations. It was hypothesized that there may be no threshold limit at which the adverse effects of lead could not occur during the course of the development of the human fetus.

In ulnar motor nerve conduction studies on 11 male volunteers fed lead acetate for 49 days, Verberk (1976) showed that there was a 13% reduction in conduction velocity in fast conducting fibers and 15% reduction in slow conducting fibers.

Table III-3. Effects of Lead Poisoning
(Beliles, 1975)

Central Nervous System

- Encephalopathy
- Fatigue
- Headache
- Tremor
- Hallucinations
- Intellectual deterioration
- Cortical atrophy
- Hydrocephalus
- Blindness
- Convulsions

Gastrointestinal

- Colic
- Loss of appetite
- Nausea
- Vomiting
- Constipation

Hematologic

- Anemia, hypochromic normocytic
- Basophilic stippling erythroblasts
- Binucleated erythroblasts
- Increased serum iron

Renal

- Hyperuricemia
- Nephritis
- Glycosuria
- Hyperaminoaciduria

Other

- Gum lead line (black or purplish line of gum margin)
- Skin pallor (ashen gray)
- Loss of Weight
- Weakness, extensor muscles (wrist or foot drop)

3. Effects on Experimental Animals

a. Acute Toxicity

No mammalian toxicological data exists for lead β-resorcylate. The only mammalian toxicological data available on lead salicylate is an oral study with rats. In this study, an LD50 of 4.3 gm/kg was determined (Potter, 1978). This LD50 value is compared to those obtained for other organic lead complexes in Table III-4. The acute toxicity of lead salicylate is similar to that of other organic lead complexes. Lead β-resorcylate should have approximately the same acute toxicity.

b. Subacute Toxicity

No specific information exists on the subacute or chronic toxicity of lead salicylate or lead β-resorcylate. However, information on other organo lead(+2) compounds may give an indication of the toxicity of lead salicylate and lead β-resorcylate. Lead acetate concentrations of 20 mg/kg/day to calves induced neurologic alterations (Wells *et al.*, 1976). Hoffmann *et al.*, (1974) found that sublethal doses of lead acetate caused ultrastructural changes in rat livers.

c. Chronic Toxicity

Numerous studies have been conducted on the effects of chronic exposure of experimental animals to lead acetate and other lead compounds. Many of these studies were aimed at behavioral effects, pharmacology, carcinogenicity, etc., of lead acetate and are reported in other sections of this report. However, Eyden *et al.* (1978) have conducted a long-term, well controlled lead acetate feeding study to determine the effects of this compound on survival and body weight in Balb/c^+ mice. These animals were fed lead acetate ranging from 0 to 4.0% of their diet. The mean survival time of the controls and mice recieving 0.5 to 4.0% dietary lead acetate are shown in Table III-5. Inspection of the table indicates a dramatic relationship between dose and survival time. Longer survival rates were observed for the 0.1% dietary level with 57% still living at the end of 541 days. The body weights of the mice receiving the 1.0% dietary level of lead acetate began to decrease significantly at 4 weeks after start of treatment as compared to the controls. At 11 weeks, these mice weighed approximately half that of the controls. Similar survivals were noted by Van Esch and Kroes (1969) for Swiss mice in their study tumor induction by lead acetate.

Table III-4. Acute Toxicity of Organic Lead Complexes to Mammals

Complex	Animal	Dose g/kg	Route of Administration	Effect	Reference
Lead salicylate	Rat	4.3	Oral	LD50	Potter, 1978
Lead naphthenate	Rat	5.1	Oral	LD50	NIOSH, 1977
Lead acetate	Rat	.204	Intraperitoneal	LDLo	"
Lead acetate	Mouse	.120	"	LDLo	"
Lead acetate trihydrate	Rat	8.5	Oral	LDLo	"
Diacetate diphenyl-lead	Mouse	.09	Oral	LD50	"
Lead tartrate	Rat	1.2	Intraperitoneal	LDLo	"

Table III-5. Relation Between Dietary Lead Acetate Dose and Mean Survival Time (Eyden *et al.*, 1978)

Lead Acetate (%)	Mean Survival Time (days)	Sex of Animal	Number of Animals
4.0	11.3 ± 1.6	F	10
3.0	18.3 ± 3.6	F	10
2.0	43.2 ± 8.25	F	10
1.0	98.7 ± 1.6	F	21
1.0	99.9 ± 1.9	M	21
0.5	115.1 ± 1.6	F	10
0	745 ± 17.2	M	150

d. Teratogenicity and Mutagenicity

Several studies have been conducted on the teratogenicity of lead acetate. Jacquet *et al.* (1975) administered a diet containing 0, 0.125, .250 and 0.500% lead acetate to female mice after mating. The mice were dissected 18 days later. The administration of lead was found to reduce markedly the incidence of pregnancies, to increase postimplantation loss, and to decrease the weight of the surviving embryos. However, no gross abnormalities were observed in the lead treated embryos. Zegarska *et al.* (1975) demonstrated that a single injection into rats of 2.5 g lead acetate/100 g body weight on the 9th day of pregnancy caused a 75% fetal mortality and a 20% incidence of developmental defects in the head of the fetuses. This study would indicate that, under these conditions of acute administration, lead acetate was teratogenic in the rat. Kennedy *et al.* (1975) treated pregnant rats and mice by gavage with doses up to 714 mg lead acetate/kg or 10 mg of tetraethyllead/kg. These lead compounds were administered daily during the period of rapid organogenesis. There were no signs of teratogenicity resulting from use of either lead compound.

Gilani (1974) studied ultrastructural changes during cardiogenesis in chick embryos administered 0.015 mg lead acetate/egg at day 2 of incubation. Abnormalities observed in the endocardial cushion were swollen mitochondria, mitochrondria with abnormal cristae and matrix, and disrupted nuclear membrane. The mitochrondria seemed to be the organelle most frequently affected. Thus, it was shown that lead poisoning can induce ultrastructural changes in developing heart.

By comparison with lead acetate, it is expected that lead salicylate and lead β-resorcylate will exhibit teratogenic effects when administered to pregnant animals in either an acute or chronic exposure.

e. Carcinogenicity

The ability of lead acetate and basic lead acetate to induce renal tumors in experimental animals has been reported by several authors (Van Esch *et al.*, 1962; Van Esch and Kroes, 1969; Waszynski, 1977). In their initial experiments with Wistar rats, Van Esch *et al.* (1962) found renal neoplasms in 13 of 24 rats fed a diet containing 1.0% (reduced to 0.5% on 92nd (M) or 115th (F) day) basic lead acetate for 2 years. Sick or dead animals were autopsied as were all animals living at the end of 2 years. Of the 1.0% group, 7 of 50 animals had renal tumors (1 renal adenoma in a female, and 2 renal adenomas and 4 renal carcinomas in males). Only 1 mouse in the 1.0 (0.5)% group had a renal tumor. However, most of these animals died early in the experiment due to basic lead acetate poisoning. In this study, hamsters were also fed basic lead acetate, however, they also died early in the experiment from basic lead acetate poisoning.

A more recent study by Waszynski (1977) confirms the earlier results on the carcinogenicity of lead acetate. This author also found the rat to be more susceptible to renal tumors as the result of lead acetate intoxication than the mouse.

f. Behavior - Symptomology

Several studies have been conducted to determine the effects of lead acetate on behavior. Behavioral responses of experimental animals is much the same as those observed in human infants. Lead salicylate and lead β-resorcylate absorption should result in the same general behavioral symptoms.

Sobotka and Cook (1975) examined the postnatal exposure of rats to lead acetate and its possible relationship to minimal brain dysfunction. In this study, rats were fed 0, 9, 27 or 81 mg lead acetate/kg body weight for 3 weeks. As weanlings, the rats displayed behavioral characteristics similar to those seen in minimal brain dysfunctional children. Thus, it would appear that exposure of rats to lead during the perinatal period may bear an etiological relationship to some of the variants of minimal brain dysfunction.

Golter and Michaelson (1975) studied the growth, behavior, and brain catecholamines in lead exposed neonatal rats. Results showed that daily oral administration of lead to newborn rats exerted no adverse effect on their body weight. They were, however, more active than age matched controls. Levels of brain dopamine were unchanged, whereas norepinephrine levels were increased. These findings would suggest a possible relationship between lead exposure during early development, increased motor activity, and brain epinephrine, and not dopamine, as had earlier been postulated.

It was pointed out (Michaelson and Sauerhoff, 1974) that the older method of studying lead encephalopathy produced in suckling rats when lead is added to the mother's diet, showed only growth retardation, and paraplegia and lesions of the cerebellum which developed during the 4th week of life. The authors, therefore, devised a new model for studying lead induced brain dysfunction in the suckling rat. In this model, they changed the mother's diet on the 6th day from one containing 5% lead acetate to one containing 25 ppm of lead and allowing the neonates free access to the same solid diet as the mother. Results showed that the sucklings had retarded body growth but did not develop paraplegia or damage of the cerebellum. However, during the 4th week, these animals did develop hyperactivity, tremors and sterotyped behavior. The authors suggested that the severe paraplegia and histopathologic lesions reported by workers using the older model obscures the signs of minimal brain dysfunction. Accordingly, they suggest use be made of this procedure as a model for studying the subtle effects of lead intoxication on the central nervous system.

Allen *et al.* (1974) showed that when infant rhesus monkeys were exposed to lead by addition of 0.5-9 mg lead acetate/kg body weight, they developed symptoms of lead poisoning within 6 weeks. The predominant changes noted were seizures, muscular tremors, and altered social behavior. In some of the more severely affected animals, visual impairment was also noted. Although visual impairment was reduced and the seizures subsided following removal of lead from the diet, the altered social behavior persisted in the infant monkey. However, in adolescent and adult monkeys, no obvious behavioral abnormalities were observed. Thus, non-human primates are similar to humans in their reactions to lead in that the infant is more susceptible to lead poisoning than the adults.

Morrison *et al.* (1975) studied the behavior of weaned mice suckled by mothers given tap water and by mothers given a 5 mg/ml of lead acetate solution during lactation. After weaning, the mice were given a choice between tap water and a lead acetate solution after lactation. Results showed that all offspring demonstrated an immediate aversion to the lead acetate solution. Further, the lead acetate offspring drank a greater quantity of total fluid (tap water plus lead acetate) after weaning than the controls. This would indicate changes in both learned and unlearned motivation for fluid after ingestion of lead via the mother's milk in infancy.

F. Environmental Effects

1. Entry into the Environment

Lead salicylate and lead β-resorcylate are manufactured in the United States for use as burning rate modifiers in solid propellant formulations. Under current operations, these lead compounds are used sporadically by Radford AAP, each with an average use rate of 670 lb/month. At full mobilization, Radford AAP would require 3,000 lb/month of each lead compound. Badger and Sunflower AAPs also use these compounds when they are operational. At full mobilization use rates of lead salicylate and lead β-resorcylate, ~45 to 150 lb/month of each compound are estimated to be lost to the New River (Kitchens *et al.*, 1978). This source of lead salicylate and lead β-resorcylate is probably the major point of entry of these compounds into the environment.

2. Behavior in Soil and Water

a. Transport, Accumulation and Degradation

Losses of lead salicylate and lead β-resorcylate to the environment will occur primarily in the Radford AAP effluents to the New River. The river concentrations which would occur at different flow rates and mixing are shown in Table III-6.

Table III-6. Estimated Levels of Lead β-Resorcylate and Lead Salicylate (in ppm) New River at Full Mobilization

Degree of Mixing	Low Flow (620 mgd)		Average Flow (2380 mgd)	
	Individual	Combined	Individual	Combined
1%	.10	.20	.03	.06
10%	.01	.02	.003	.006
100%	.001	.002	.0003	.0006

The table assumes the lead salicylate and lead β-resorcylate are initially in the water. Weitzel *et al.* (1976) found lead levels at Radford AAP in the New River ranged from 1-2 ppb. However, sediment levels were greater than 100 ppm. These data indicate that lead β-resorcylate and lead salicylate accumulated in the sediment of the New River.

Once in the sediment, it is probable that the lead compounds are slowly degraded either biologically or through complexation by the sediment. This complexation should be similar to that observed with organo-lead compounds in soil. A pathway for complexation of organo lead compounds in soil is shown in Figure III-6. The end result of this pathway is generally immobilization of the lead as insoluble carbonate or phosphate salts. Lead

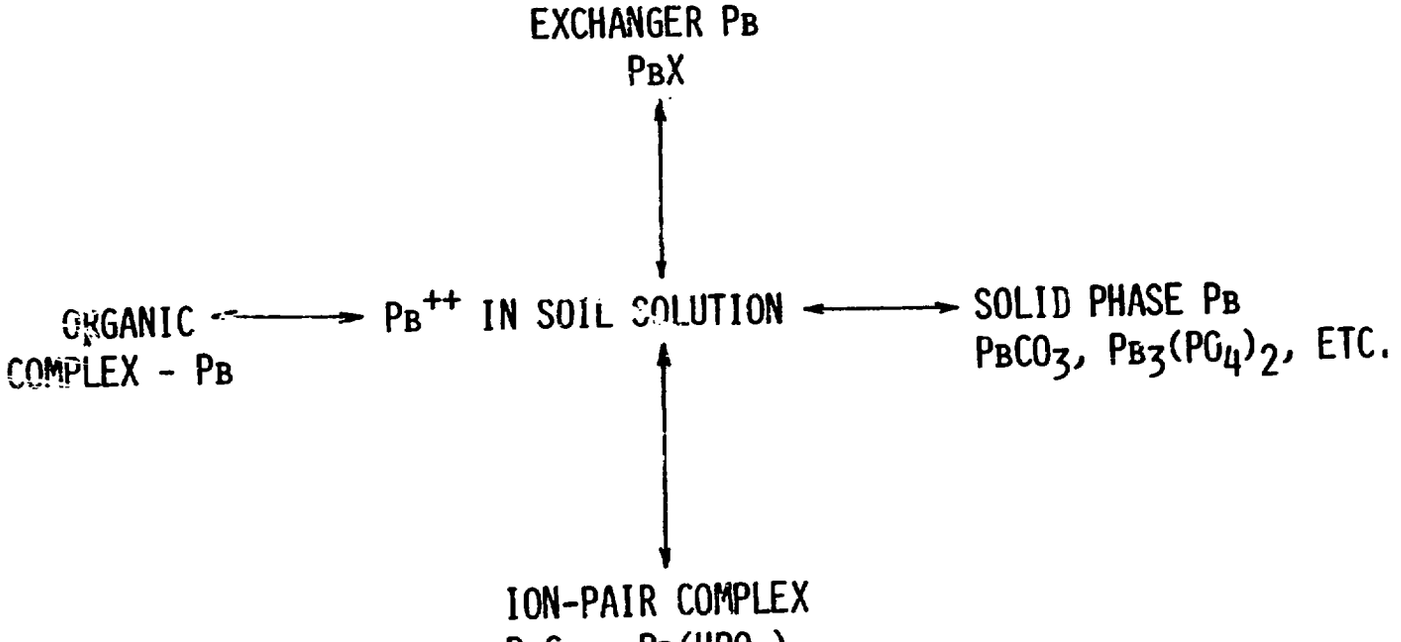


Figure III-6. Complexation of Organo lead Compounds in Soil (Wentsel, R. from data in Jurinak and Santillan-Medrano, 1974)

salicylate and lead β-resorcylate are expected to undergo this type of reaction. The controlling factors for lead fixation appears to be pH and cation-exchange capacity (Zimdahl and Skogerboe, 1977).

b. Background Concentrations

Although natural lead salicylate and lead β-resorcylate concentrations in the environment are non-existent, lead background concentrations have been studied. Swaine (1955) estimated typical levels of lead in soil ranged from 1-200 ppm with a mean of 15 ppm. Kopp and Kroner (1967) sampled 1500 streams and found lead levels greater than 10 ppb in 20% of the samples. Livingstone (1963) estimated natural lead levels in water to be between 1-10 ppb.

3. Effects on Animals

a. Mammals

The effects of compounds related to lead salicylate and lead β-resorcylate on experimental mammals were discussed in Section D.3. No information on the effects of environmental exposure to lead salicylate, lead β-resorcylate or related compounds to mammals was found.

b. Birds

No information on the effects of lead salicylate, lead β-resorcylate or related compounds on birds was found.

c. Fish

No information on the toxicity of lead salicylate or lead β-resorcylate to aquatic life was found in the literature. A large amount of aquatic toxicological information on inorganic lead compounds and organolead compounds exists, however, the inorganic lead and organolead compounds do not possess the same type of bonding and/or valency as lead salicylate and lead β-resorcylate. The only compound with similar bonding for which aquatic toxicity information exists is lead acetate. However, lead acetate is more soluble and hydrolyzes more readily than lead salicylate or lead β-resorcylate. Thus, the toxicity of lead salicylate and lead β-resorcylate is expected to be lower than that observed for lead acetate due to the lower concentration of ionic lead.

The effects of exposure of various fish species to inorganic lead, lead acetate and organolead are presented in Table III-7. As can be observed from the table, lead acetate has approximately the same toxicity as lead chloride in soft water. Lead acetate is soluble but not completely ionized whereas lead chloride is only sparingly soluble. Lead salicylate and lead β-resorcylate would be expected to be less toxic than lead acetate with an estimated LC50 of 15-20 ppm in soft water and >500 ppm in very hard water.

Table III-7. Toxicity of Various Lead Compounds to Fish

Organism	Compound	Type of Test	Hardness (ppm $CaCO_3$)	pH	Temperature °C	Lead Levels (ppm)	Effects	Reference
Fathead minnow (*Pimephales promelas*)	$PbCl_2$	acute static	20 360	7.5 8.2	25 25	5.8-7.33 .482	96-hr LC50 96-hr LC50	Pickering and Henderson, 1965
Fathead minnow (*P. promelas*)	$PbCl_2$ H_2O	acute static	20	7.5	25	7.48	96-hr LC50	Pickering and Henderson, 1965
Bluegill Sunfish (*Lepomis macrochirus*)	$PbCl_2$	acute static	20 360	7.5 8.2	25 25	23.8 442	96-hr LC50 96-hr LC50	Pickering and Henderson, 1965
Bluegill Sunfish (*L. macrochirus*)	$(C_2H_5)_4Pb$	acute static	20	6.9-7.5	20	84-163	48-hr LC50	Turnbull *et al.*, 1954
Bluegill Sunfish (*L. macrochirus*)	Pb salt	chronic (E-L)	41	6.7-7.2	-	0.7-.12	MATC	Sauter *et al.*, 1976
Rainbow trout (*Salmo gairdneri*)	$Pb(NO_3)_2$	acute static	353	6.89-8.78	7	471	96-hr LC50	Davies, 1976
Rainbow trout (*S. gairdneri*)	$Pb(NO_3)_2$	acute flow through	43-45	7.15-7.5	14.7	3.75	24-hr LC50	Benoit and Holcombe
Rainbow trout (*S. gairdneri*)	Pb salt	chronic (E-L)	35	6.9-7.4		.071-.146	MATC	Sauter *et al.*, 1976

E-L — Embryo Larvae

MATC — Maximum Acceptable Toxicant Concentration

This difference in toxicity of lead compounds in different waters is due to precipitation of lead as insoluble lead carbonate in hard water. Thus the lead is unavailable to the aquatic organisms. The effects of any lead compounds on aquatic organisms is thus determined to a large extent by the hardness of the water. The New River which receives effluents from Radford AAP has soft water, ~ 20-40 ppm as $CaCO_3$ (Weitzel *et al.*, 1976). Thus, all lead compounds are expected to be relatively toxic to aquatic life of this river.

Bioconcentration factors for lead in brook trout and the bluegill have been reported to be 42 and 45, respectively (Holcombe *et al.*, 1976; Atchison *et al.*, 1977). No bioconcentration factors for lead salicylate or lead β-resorcylate were found in the literature. Sufficient data were also not available to calculate an octanol-water partition coefficient.

d. Amphibians

No information was found on the effects of lead salicylate, lead β-resorcylate or related compounds to amphibians.

e. Invertebrates

No information is available on the toxicity of lead salicylate and lead β-resorcylate to invertebrates. However, the data in Table III-8 show the toxicity of lead acetate to *Daphnia magna*. Lead acetate was found to be moderately toxic with a ECL_0 of 1.1 ppm.

Table III-8. Toxicity of Lead Compounds to *Daphnia magna*

Compound	Hardness (ppm as $CaCO_3$)	pH	Result	Concentration (ppm)	Reference
Lead acetate	16	7.6	48 hr EC50	2.5	Bringmann and Kuhn (1977)
Lead acetate	11	7.6	48 hr ECL_0	1.1	Bringmann and Kuhn (1977)

f. Microorganisms

No information was found on the toxicity of lead salicylate or lead β-resorcylate to microorganisms or degradation of these compounds by microorganisms. However, lead acetate inhibited cell replication of *Pseudomonas putida* at 1.8 ppm (Bringmann and Kuhn, 1976).

4. Effects on Plants

No information was found on the interaction of lead salicylate or lead β-resorcylate with plants. Lead acetate was found to inhibit cell multiplication of the algae, *Microcystis aeruginosa* at 0.45 ppm (Bringmann and Kuhn, 1976.)

G. Regulations and Standards

1. Air and Water Standards

There are no air or water standards specific for lead salicylate or lead β-resorcylate. However, criteria have been set for lead in potable water (Federal Register, 1979.) For protection of human health, the lead content of potable waters can be no greater than 50 µg/l. Proposed freshwater critera for protection of aquatic life have been revised for lead taking into account water hardness (Federal Register, 1979). The equation for the 24-hour average concentration is 1.51 ln (hardness) - 3.37. The maximum allowable concentration is 1.51 ln (hardness) - 1.39.

2. Human Exposure Standards

No specific standards for lead salicylate or lead β-resorcylate have been set for occupational exposure to this chemical. However, a criteria document has recommended an air standard of 150 µg(Pb)/m^3 for lead stearate (NIOSH, 1977). A similar TLV for lead salicylate or lead β-resorcylate should afford workers adequate protection.

H. Evaluation and Comments

1. Lead β-Resorcylate

Very little is known concerning the chemistry and toxicological and environmental hazards of lead β-resorcylate. No information on the acute and chronic mammalian toxicology of this salt is available. The biological interactions of lead β-resorcylate can only be inferred from studies on lead acetate. Aquatic toxicity information on lead β-resorcylate is non-existant.

No information is available on the environmental fate of lead β-resorcylate. Hydrolysis of this salt to inorganic lead and bioaccumulation are both possible.

In view of the very limited data available on lead β-resorcylate, the following studies are recommended to fill in the information gaps.

1. Further chemical analysis to identify the specific lead compounds and their percentage composition in lead β-resorcylate used in propellants. This work should include further enumeration of the chemistry of lead β-resorcylate and development of a scheme to qualitatively identify and quantitate lead β-resorcylate and breakdown products in the environment.

2. Sampling and analysis of lead β-resorcylate concentrations entering the New River in Radford AAP effluents and correlation of these results with production. The concentrations of these salts and breakdown products in the sediments and biota of the New River should also be determined.

3. The LD50 of lead β-resorcylate to rats should be determined.

4. A chronic feeding study using rats should be undertaken to determine the long term effects and carcinogenic potential of lead β-resorcylate.

5. Aquatic toxicity tests in water of similar chemical composition to the New River should be indicated to determine the toxicity of lead β-resorcylate to fish and invertebrates. These tests should include 96-hr static LC50 and 30-day flow-through chronic tests.

6. Effectiveness of the proposed treatment facilities at Radford AAP in removing this salt from the effluent should be evaluated.

2. Lead Salicylate

Information on the chemistry and toxicological and environmental hazards of lead salicylate is extremely limited. The data that do exist on the chemical properties of this complex are conflicting. It appears that hydrolysis will be the major environmental reaction although substitution has also been shown to occur.

The only toxicological data available are an oral LD50 to rats. All information presented on the biological interaction of lead salicylate has been inferred from the data available on lead acetate. From these limited data, it appears that lead salicylate has a low toxicity to mammals when administered in an acute dose. However, no information exists for chronic exposure to this complex.

The toxicity of lead salicylate to aquatic species is hard to predict from the data available, but it is probably less toxic than lead acetate. No data are available on the bioaccumulation potential of lead salicylate.

Further information is needed in order to reliably assess the toxicological and environmental hazards of lead salicylate. Therefore, the following studies are recommended in order to obtain these data.

1. Gather further information on the physical and chemical properties of lead salicylate. Initial investigation should center on the behavior of lead salicylate in aqueous solutions.

2. A chronic feeding study with rats should be undertaken to determine the long term effects of lead salicylate including its carcinogenic potential.

3. An acute skin absorption test should be carried out on rabbits or guinea pigs in order to determine the ability of lead salicylate to penetrate the skin.

4. Aquatic toxicity tests in water similar in chemical composition to the New River should be conducted to determine the toxicity of lead salicylate to fish and

invertebrates. These tests should include 96-hour static LC50 and 30-day flow-through chronic tests.

5. Further studies which may be of value depending on the outcome of 1 and 4 include:

   - sampling and analysis of Radford AAP effluents and sediment and biota of the New River;

   - determine the ability of the proposed treatment facilities to effectively remove lead salicylate form the Radford AAP effluent.

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PROBLEM DEFINITION STUDY ON
2-NITRODIPHENYLAMINE

SUMMARY

2-Nitrodiphenylamine is a stabilizer used by the Army in the manufacture of solid propellants. Currently Radford AAP is the only Army Ammunition Plant producing propellants containing 2-nitrodiphenylamine. This compound enters the environment in the wastewater from the nitroglycerin manufacture and propellant formulation areas. The amount entering the New River during full mobilization operations at Radford AAP is estimated at 80-200 lb/month.

2-Nitrodiphenylamine has a low acute toxicity to mammals. Metabolism to 4-hydroxy- and 4,4'-dihydroxy-2-nitrodiphenylamine and rapid elimination from the body similar to diphenylamine is likely. N-hydroxyl-2-nitrodiphenylamine is also postulated as a metabolite based on the appearance of methemoglobin in the blood.

In the aquatic environment, 2-nitrodiphenylamine is probably toxic to fish and invertebrates in the low ppm concentration range. This inference is based on the aquatic toxicity data available on compounds similar to 2-nitrodiphenylamine since no data are available on this compound. From the limited amount of information available, 2-nitrodiphenylamine appears to be non-toxic to microorganisms. A mixed culture can use this compound as a sole carbon source.

The following studies are recommended in order to obtain the needed information to fully determine the environmental hazards of 2-nitrodiphenylamine in the Radford AAP effluent:

- studies to determine the water solubility and octanol-water partition coefficient of 2-nitrodiphenylamine
- sampling and analysis of Radford AAP's effluent for 2-nitrodiphenylamine and correlation of effluent concentrations with production data
- biodegradation studies on 2-nitrodiphenylamine
- detailed aquatic toxicity studies on 2-nitrodiphenylamine

FOREWORD

A. Study Goals

This report presents the results of an evaluation of the available information on the toxicological and environmental hazards of 2-nitrodiphenylamine. 2-Nitrodiphenylamine is a stabilizer used in propellant formulations. This compound enters the environment in wastewater generated during nitroglycerine manufacture and propellant formulation at Army Ammunition Plants. The Army is one of the main users of 2-nitrodiphenylamine and propellant manufacturing is one of the main sources of pollution of this compound. This evaluation of the toxicological and environmental hazards of 2-nitrodiphenylamine was undertaken in order to aid the Army in identification of research needs and in recommendation of effluent criteria for this compound.

B. Study Methodology

The methodology utilized to gather information for this report included a detailed search of the literature and numerous personal contacts. During the literature search, the following sources were reviewed for pertinent information on 2-nitrodiphenylamine:

- Chemical Abstracts	1940 - present
- Biological Abstracts	1950 - present
- Excerpta Medica	1950 - present
- TOXLINE	1965 - present
- National Technical Information Service	1964 - present
- Defense Documentation Center	1958 - present
- COMPENDEX	1970 - present

The search of the literature revealed very little information on 2-nitrodiphenylamine.

Personal contacts were made with U.S. and foreign manufacturers of 2-nitrodiphenylamine; Army Ammunition Plant personnel and Army and civilian researchers. The specific contacts made and results are presented below.

1. U.S. Manufacturer

American Cyanamid Company is the only U.S. manufacturer of 2-nitrodiphenylamine. Three people were contacted within American Cyanamid:

Dr. Pinto
Mr. R. W. McCullough
Mr. Keith Baarson

Dr. Pinto was contacted Sept. 18, 1978 and said that Mr. McCullough would have material and safety data sheets on 2-nitrodiphenylamine. Mr. McCullough was contacted Sept. 22, 1978. He indicated that Keith Baarson, assistant administrator to the Director of Toxicology, would have details of their mammalian toxicity tests. Mr. Baarson was contacted and one piece of information on the sublethal effects of 2-nitrodiphenylamine was received.

2. Foreign Manufacturers

Four foreign companies listed in the 1978 Directory of Chemical Producers in Western Europe as 2-nitrodiphenylamine producers were contacted by Telex in October, 1978. These companies were:

FED. REP. OF GERMANY
Bayer AG

UNITED KINGDOM
Hickson & Welch Ltd.
Hopkin & Williams
Koch-Light Laboratories Ltd.

None of the companies responded.

3. Radford AAP Personnel

The following Radford AAP personnel were contacted Sept. 28, 1978:

Mr. John Horvath
Mr. Tom Grady
Mr. Ted Topper

Mr. Horvath had no information. Mr. Grady suggested calling the manufacturer. Mr. Topper searched for toxicological information but could not find any.

4. Other Sources

Dr. Jay Abercrombie of the U.S. Army Chemical System Laboratory, Aberdeen Proving Ground, MD, was contacted Sept. 12, 1978. He reported no information on 2-nitrodiphenylamine.

Mr. J. Gareth Pearson of USAMBRDL, Fort Detrick, MD, was visited in September, 1978. However, no data were available on the aquatic toxicology of 2-nitrodiphenylamine.

Dr. W. G. Charackles of Rice University, Houston, Texas was contacted concerning a study he performed on the waste treatment of Otto Fuel II containing 2-nitrodiphenylamine. The study he performed was a small one and no additional data over that already published are available.

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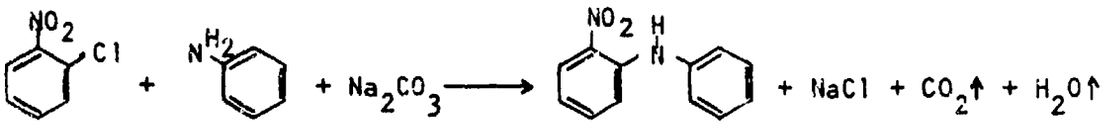
IV. 2-Nitrodiphenylamine

A. Alternate Names

2-Nitrodiphenylamine is a secondary aromatic amine which has civilian applications as a dyestuff and military application as a stabilizer in double base propellant formulations. It is produced commercially by American Cyanamid Co. by the reaction of 2-chloronitrobenzene and aniline in the presence of sodium carbonate. A yield of 96% is obtained by removing water in the form of its aniline azeotrope (Desseigne and Rabussier, 1957):



2-Nitrodiphenylamine has a molecular formula of $C_{12}H_{10}N_2O_2$ and a molecular weight of 214.23. The pertinent alternate names for 2-nitrodiphenylamine are listed below:

CAS Registry No.:	119-75-5
CA Name (9CI):	Benzenamine, 2-nitro-N-phenyl-
CA Name (8CI):	Diphenylamine, 2-nitro-
Wiswesser Line Notation:	WNR BMR
Synonyms:	2-nitrodiphenylamine; o-nitrodiphenylamine; o-nitro-N-phenylaniline; SUDAN YELLOW 1339; C.I. 10335

B. Physical and Chemical Properties

1. Physical Properties

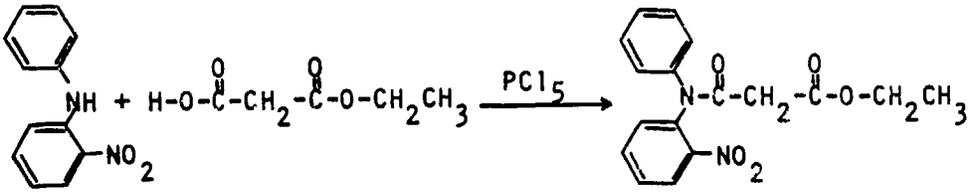
The physical properties of 2-nitrodiphenylamine are listed in Table IV-1. The infrared, NMR and ultraviolet spectra are shown in Figures IV-1 through IV-3. The mass spectrum of 2-nitrodiphenylamine has significant peaks at m/e = 214 (m+), 197, 180, 167, and 77 (Budzikiewicz *et al.*, 1972; Peters, 1973).

The interplanar spacings and the intensities of the X-ray diffraction lines are: 10.27(s), 8.47(s), 5.98(m-), 5.34(s), 4.83(m), 4.51(s+), 3.96(m), 3.73(f), 3.58(f-), 3.45(m), 3.26(m-), 3.18(m), 3.08(vf), 2.98 (f broad), 2.82 (f+ broad), 2.73(vf), 2.66(vvf), 2.50(vf+), 2.10(vf), 2.05(vf), 1.94(vf), 1.86(vf), 1.77(vvf), 1.62(vf) (s = strong, v = very, m = medium, f = faint) (Soldate and Noyes, 1947).

2. Chemical Properties

a. General Chemistry

The chemistry of 2-nitrodiphenylamine involves the amine and nitro functional groups as well as the benzene rings. The basicity of 2-nitrodiphenylamine is lower than that of diphenylamine due to the delocalization of the non-bonded nitrogen electrons into the aromatic rings and nitro group (Varkley *et al.*, 1974). The amino functional group will react with acid chloride to form amides (Roussel-Uclaf, 1970).



Tertiary amines are formed with arylhalides in the presence of carbonate (Desseigne and Rabussier, 1957; Bacon and Hamilton, 1972)

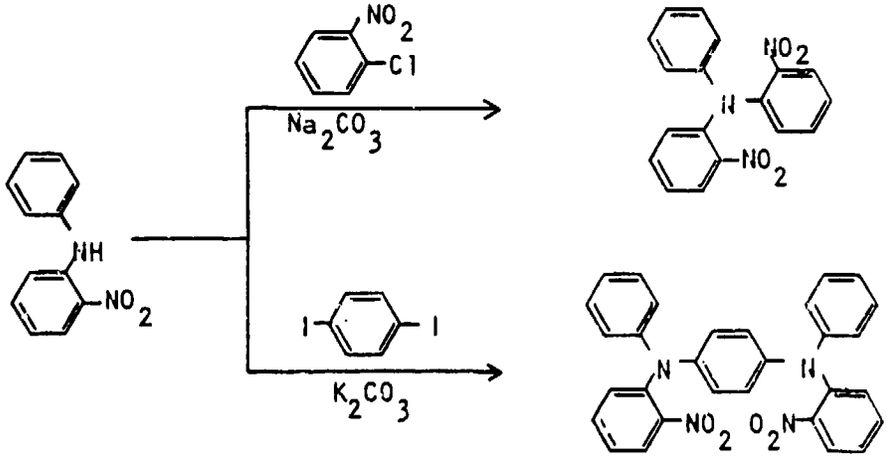


Table IV-1. Physical Properties of 2-Nitrodiphenylamine

Physical Form @ 20°C:	solid
Crystalline Form:	orthorhombic
Melting Point:	75-76°C
Boiling Point:	223 @ 20 mm Hg
Volatility:	no data available
Vapor Pressure:	no data available
Specific Gravity:	1.366
Octanol-Water Partition Coefficient:	no experimental data available: calculated value = 3.07
Solubility:	water - soluble only at low levels; no specific numbers available
	ethanol - 1 g/100 g @ 0°C 2 g/100 g @ 25°C 15 g/100 g @ 50°C 35 g/100 g @ 62°C 90 g/100 g @ 65°C
	methanol - 2.4 g/100 g @ 20°C
	acetone - 43.6 g/100 g @ 20°C
	benzene - 51.7 g/100 g @ 20°C

*CRC, 1973; American Cyanamid, 1976a; Hawley, 1976; Prasad and Shanker, 1936; Desseigne and Rabusser, 1957.

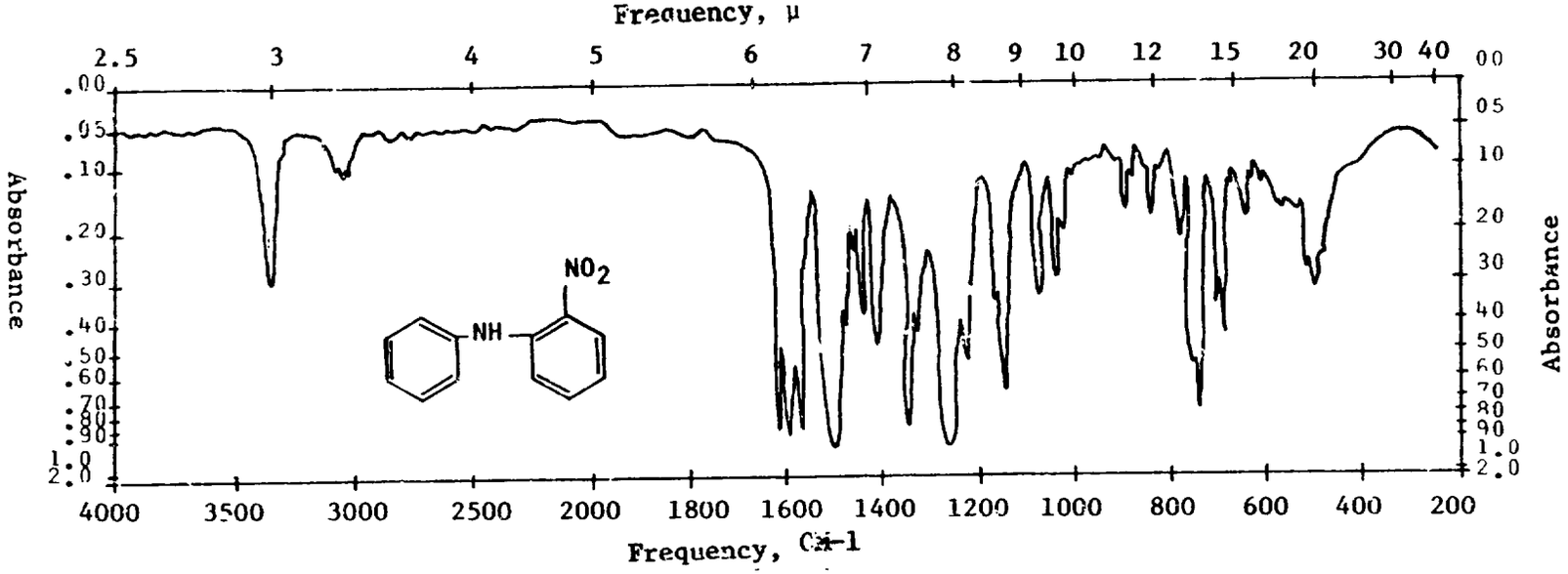


Figure IV-1. Infrared Spectrum of 2-Nitrodiphenylamine (© Sadtler Research Laboratories, Inc., 1969)

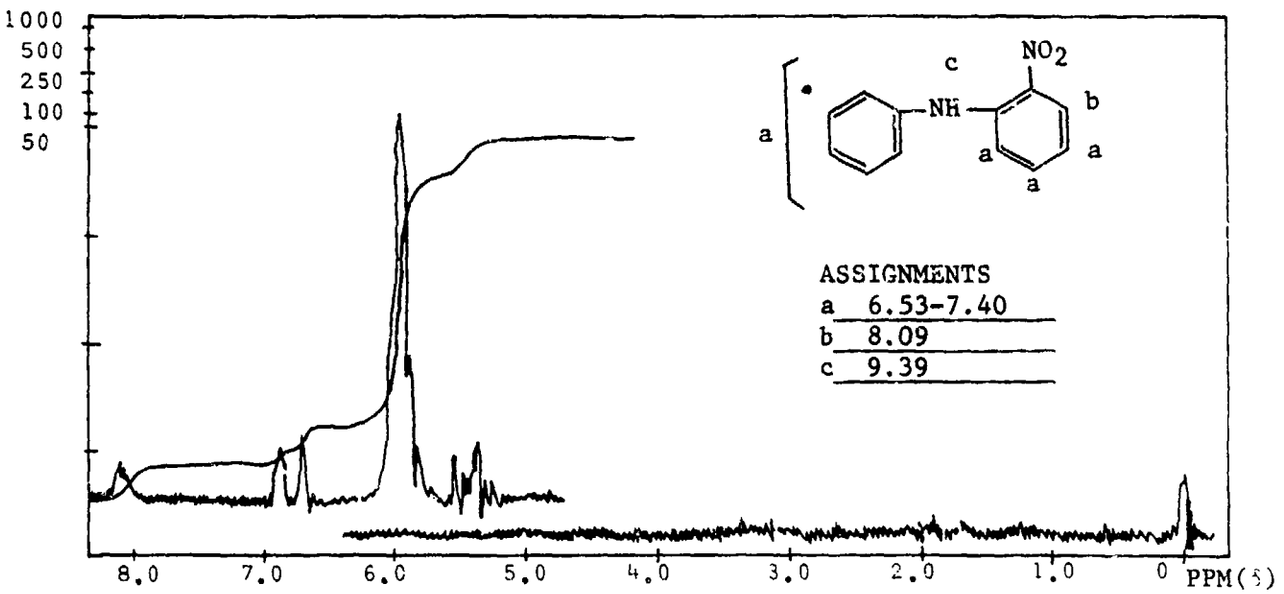


Figure IV-2. Nuclear Magnetic Resonance Spectrum of 2-Nitrodiphenylamine.
(© Sadtler Research Laboratories, Inc., 1967)

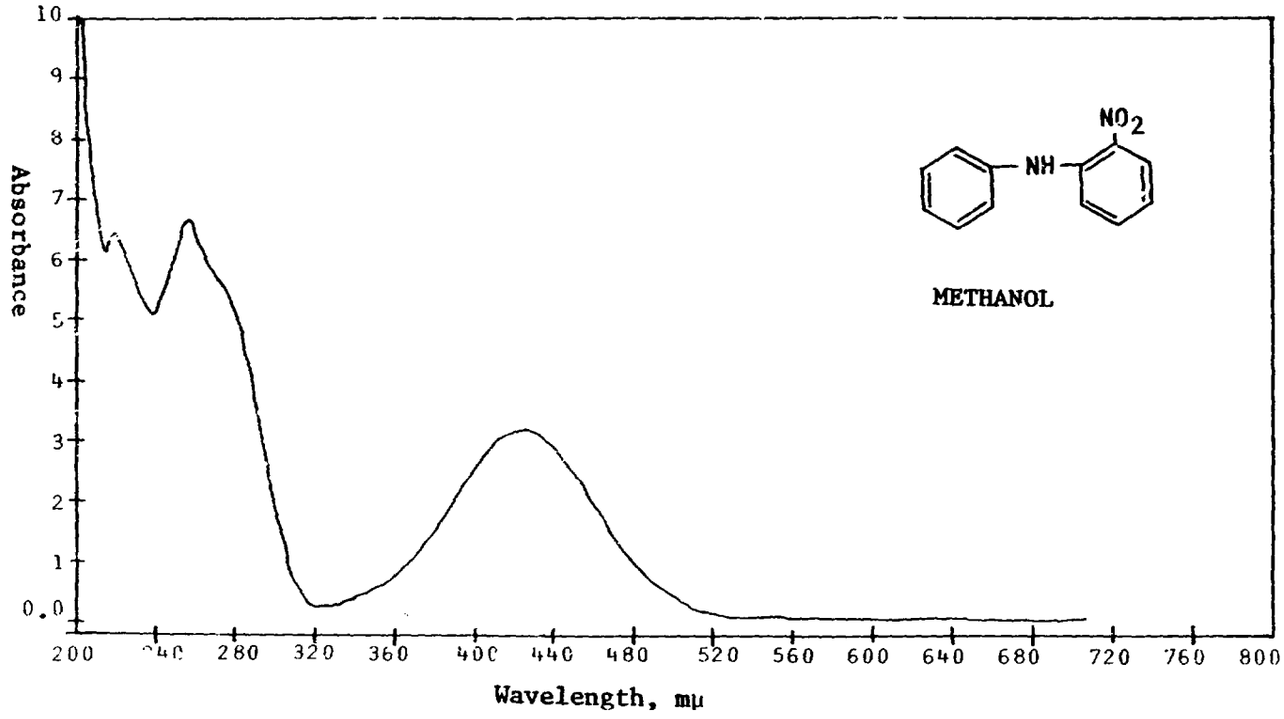
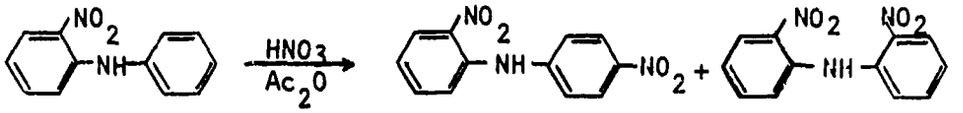
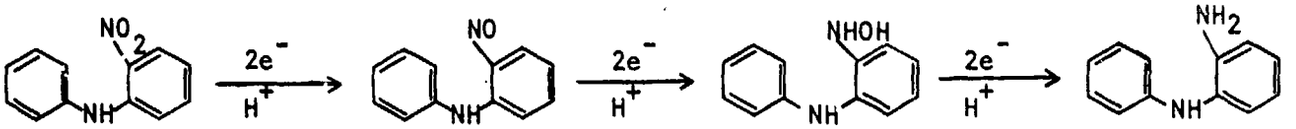


Figure IV-3. Ultraviolet - Visible Spectrum of 2-Nitrophenylamine in Methanol. (©Sadtler Research Laboratories, Inc., 1969)

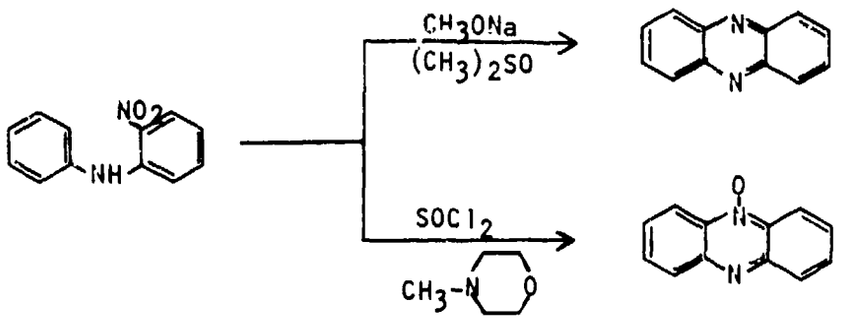
Nitration of the benzene ring in acetic anhydride proceeds well, although somewhat slower than diphenylamine (Sharnin and Falyakhov, 1976).



The nitro group is easily reduced via polarography yielding 2-aminodiphenylamine with intermediate hydroxylamine species being proposed (Varkey *et al.*, 1974).



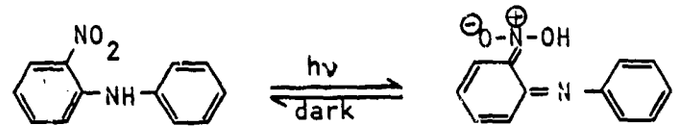
2-Nitrodiphenylamine has been cyclized yielding phenazene (Wataya *et al.*, 1975a) and phenazine oxides (Wataya *et al.*, 1975b).



b. Environmental Chemistry

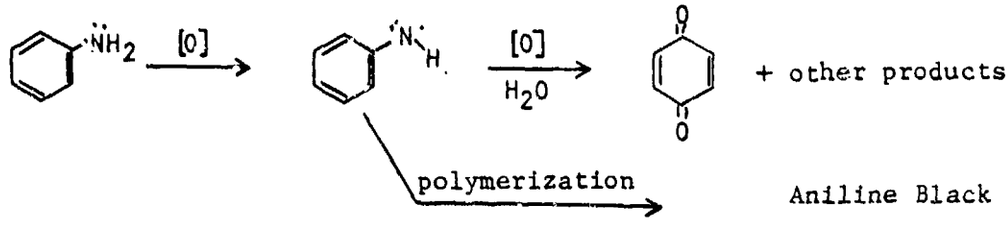
No studies of the environmental chemistry of 2-nitrodiphenylamine have been reported in the literature. The molecule is not likely to be subject to acid/base hydrolysis of any kind, therefore, the most likely degradation pathways would be via photochemical or oxidation/reduction reactions.

2-Nitrodiphenylamine can undergo photochromic enolization:

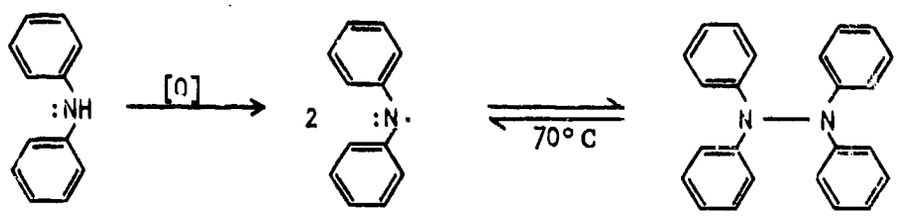


This reaction is reversible, but would be the first step of other complex reaction mechanisms. The photodegradation of 2-nitrodiphenylamine has been studied and shown to be significantly slower than the degradation of the 3-and 4-nitro isomers (Asquith and Williamson, 1969). The reaction proceeds more rapidly in neutral ethylacetate-ethanol-water than in similar acidic or basic solutions. In ethyl acetate alone, the photodegradation reaction of 2-nitrodiphenylamine occurs at a slower rate than in the ethylacetate-ethanol-water system. The presence of oxygen has been shown to inhibit degradation by quenching the triplet state. The presence of hydrogen donors enhances the process, thus, suggesting a reductive process. The participation of quinoidal forms has been suggested (Asquith *et al.*, 1976). Reduction or photoreduction would likely yield 2-aminodiphenylamine analogous to the previously mentioned polarography results (Varkey *et al.*, 1974).

2-Nitrodiphenylamine as well as the potential photoproduct, 2-aminodiphenylamine, are likely to be susceptible to air oxidation. Aniline, diphenylamine, and aromatic amines in general are subject to air oxidation, and molecules with electronegative substituents have increased reactivity (Fieser and Fieser, 1961; Streitwieser and Heathcock, 1976). Generally unidentified colored materials are formed. The oxidation of aniline under varying oxidizing conditions can lead to a host of products including azobenzene, azoxybenzene, nitrobenzene, quinone, Aniline Black and others. The initial step in all cases appears to be the abstraction of the hydrogen atom leading to a nitrogen radical:

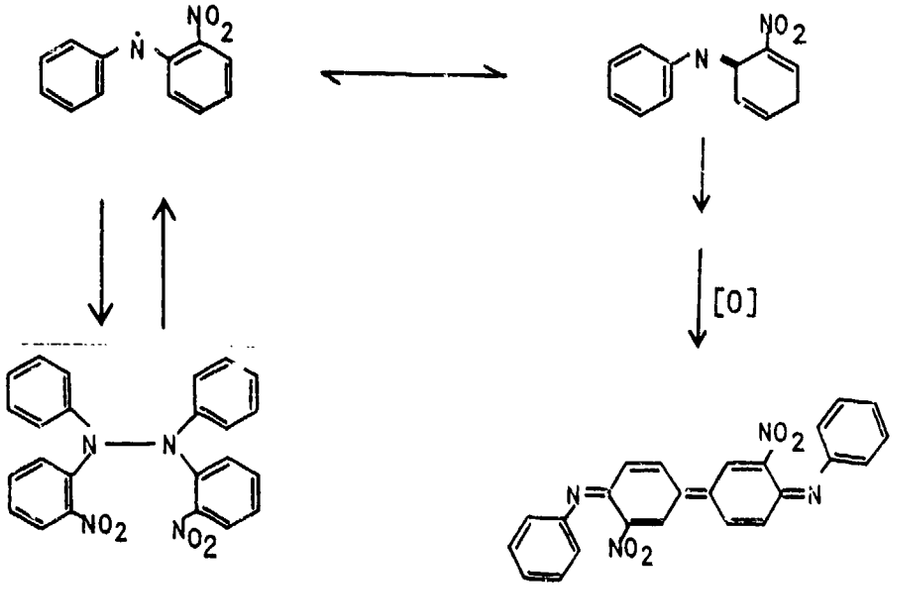


The radical formed from diphenylamine is more resistant to further reactions than the corresponding aniline radical. However, the diphenylamine radical has been shown to undergo reversible dimerization (Fieser and Fieser, 1961):



Both the dimer and the radical will undergo further reactions in the presence of radical scavengers. 4-Aminodiphenylamine has been suggested as a potential intermediate in the oxidation of aniline to benzoquinone. Since 2-aminodiphenylamine is a likely photoreduction product of 2-nitrodiphenylamine, an analogous reaction is possible for 2-nitrodiphenylamine.

Oxidative coupling, similar to the polymerization of aniline to form Aniline Black, is possible. Dimerization via N-N coupling has already been mentioned and C-C coupling is likewise feasible, leading to numerous possibilities.



C. Monitoring and Analysis

1. Analytical Methods

Analytical techniques have been developed for the determination of 2-nitrodiphenylamine in propellant compositions as well as in river sediment samples. Preliminary preparation generally involves extraction with methylene chloride, ether or benzene.

Qualitative tests for 2-nitrodiphenylamine include colorimetric spot tests with alcoholic NaOH, NH_3, NaCN, and conc. H_2SO_4 (Davis and Ashdown, 1924), column chromatography and thin layer chromatography (Schroeder *et al.*, 1949; Archer, 1975; Marvillet, 1958; Del Campo, 1975; Grindlay, 1973; Asquith and Williamson, 1969; and Landram *et al.*, 1970).

Several methods which have been developed for quantitation of 2-nitrodiphenylamine are summarized in Table IV-2. These methods include infrared spectroscopy using absorbances at about 6.26 microns (Wagner *et al.*, 1967); ultraviolet-visible absorption at 420 nm (Mil-STD-286B Method 218.4.3, 1967); oxidation with ferric ion to a colored quinoneimide which can be determined by its absorbance at 530 nm (DeAtley, 1970); and a volumetric bromination technique (Marvillet and Tranchant, 1957; Mil-STD-286B Method 218.1.2, 1969). Gas chromatography has been employed by numerous researchers, some utilizing low injection port temperatures to avoid reactions between nitroglycerine and 2-nitrodiphenylamine in the injector port (Trowell and Philpot, 1969; Trowell, 1970). Some researchers removed the nitroglycerine prior to analysis (Weitzel *et al.*, 1976). A variety of column packings were employed including 5% OV-17 on Chromosorb Q (Trowell and Philpot, 1969), 2.5% OV-17 and 2.5% QF-1 on Chromosorb Q (Trowell, 1970), 5% Dexsel 300 on HPCW (Weitzel *et al.*, 1976) and 3.8% OV-101 or 1.1% OV-225 and 2.5% OV-210 on Chromosorb W (Dykes and Alley, 1974; Alley and Dykes, 1973). Oven temperatures were generally varied between 70 and 250°C.

High pressure liquid chromatography, utilizing ultraviolet detection at 254 nm has also been employed for the analysis of 2-nitrodiphenylamine. Solvent systems have include methanol/water on a Microbondapak C-18 column (Poyet *et al.*, 1976), methylene chloride/cyclohexane on a Corasil II column (Doali and Juhasz, 1975) and dioxane/hexane on a Microporasil column (Weitzel *et al.*, 1976).

2. Monitoring

2-Nitrodiphenylamine has been monitored in effluent water as well as core sediment samples taken from the New River at Radford AAP (Weitzel *et al.*, 1976). Effluent water ranged from 1-14 ppm with a mean of 3.5 ppm while sediment samples ranged from 0.5 to 12.2 ppm with a mean of about 1.5 ppm.

Table IV-2. Methods for Quantitative Analyses of 2-Nitrodiphenylamine

Method	Sample Preparation	Analytical Conditions	Concentration Limits	Reference
Infrared	Extract with ether, evaporate and dissolve in CCl_4.	Optical density measured at 7.87μ. Contribution of dinitrotoluene is deducted in calculation.	Not specified, 800 ppm investigated	Wagner *et al.*, 1967
Ultraviolet	Extract with CH_2Cl_2, evaporate, dissolve in acetic acid and dilute with ethanol.	Maximum absorbance between 420 and 430 nm is measured. Concentration obtained from calibration curve.	5-15 ppm	Mil-STD-286B Method 218.4.3, 1967
Volumetric Bromination	Extract with CH_2Cl_2, evaporate, dissolve in acetic acid, and add KI and either CH_2Cl_2 or CCl_4.	Brominate with potassium bromate/bromide and HCl. Titrate unused reagent with $Na_2S_2O_3$ until I_2 color disappears.	Not specified, 150 mg investigated	Marvillet and Tranchant, 1957; Mil-STD-286.B Method 218.1.2, 1969.
Gas Chromatography	Extract with CH_2Cl_2.	Column: 4' 5% OV-17 on GAS CHROM Q Oven: 70-250°C @ 15°/min Flow: 15 cc/min Detector: FID	Not specified ~ 8 ppm studied	Trowell and Philpot, 1969
"	Extract with CH_2Cl_2.	Column: 6' 2.5% OV-17, 2.5% QF-1 on GAS CHROM Q Oven: 70-230°C @ 10°/min Flow: 65 cc/min Detector: FID	Not specified	Trowell, 1970
"	Extract with Benzene. Remove nitroglycerine via HPLC if 100 fold greater than 2-NDPA.	Column: 6' 5% DEXSIL 300 on HPCV Oven: 115-250°C @ 30°/min Flow: 50 cc/min Detector: FID	0.5 ppm	Weitzel *et al.*, 1976

Table IV-2 (Cont'd.). Methods for Quantitative Analyses of 2-Nitrodiphenylamine

Method	Sample Preparation	Analytical Conditons	Concentration Limits	Reference
Gas Chromatography	Extract with CH_2Cl_2	Column: 3.8% OV-101 or 1.1% OV-225 on Chromosorb W Detector: FID	Not specified	Dykes and Alley, 1974
Gas Chromatography	Extract with CH_2Cl_2	Column: 3.8% OV-101 on Gas-Chrom Q 2.5% OV-210 on Chromosorb W-HP 1.1% OV-225 on Gas-Chrom Q Detector: FID	Not specified > 0.1% investigated	Alley and Dykes, 1973.
High Performance Liquid Chromatography	Extract with $CHCl_3$	Column: MicroBondapack C 18 Mobile Phase: 67.5% Methanol/water Flow: 1 ml/min Detector: UV @ 254	Not specified	Poyet *et al.*, 1976
"	Extract with CH_2Cl_2	Column: CORASIL II Mobile Phase 20% CH_2Cl_2/cyclohexane Flow: 0.5 ml/min Detector: UV @ 254	33-667 ppm	Doali and Juhasz, 1975
"	Extract with Benzene	Column: Microporasil Mobile Phase: 10% dioxane/hexane Flow: 2 ml/min Detector: UV @ 215	> 10 ppm	Weitzel *et al.*, 1976

D. Health Effects

1. Biology

Very little information exists on the biology of 2-nitrodiphenylamine. Predictions of the biological interactions of this compound must be made by analogy to related compounds such as diphenylamine.

2-Nitrodiphenylamine may be absorbed through the gastrointestinal or respiratory tracts by ingestion or inhalation, respectively. The percentage actually absorbed through either of these routes is not known. Some absorption may also occur through the skin (American Cyanamid, 1976b).

Aromatic amines are known to be metabolized *in vivo* by oxidative attack. The major metabolites are aminophenols (Williams, 1959; Alexander *et al.*, 1965) and/or hydroxylamines (Boyland and Booth, 1962; Uehleke, 1963). The metabolism of diphenylamine in the rat, rabbit, and man was studied by Alexander *et al.* (1965). In this study, the main diphenylamine metabolites in urine were identified as 4-hydroxydiphenylamine and 4,4'-dihydroxydiphenylamine. These metabolites were found in human urine after ingestion of 100 mg of diphenylamine, in rat urine after intraperitoneal injection of 5 mg diphenylamine and in rabbit urine after ingestion of five one gram doses over nine days. In addition, a minor metabolite, 2-hydroxydiphenylamine was found in rabbit urine. Free diphenylamine was found in rabbit and human urine, but not in rat urine. Alexander *et al.* (1965) found no N-hydroxydiphenylamine in any urine samples, even after intraperitoneal injection of that compound into the rat.

The formation of methemoglobin following administration of aromatic amines has been attributed to the *in vivo* formation of N-hydroxy compounds (Uehleke, 1963). Alexander *et al.* (1965) administered an oral dose of diphenylamine (1 mM/kg in aqueous suspension) to a 2 kg cat. Blood samples showed methemoglobin formation equivalent to that produced by the same dose of acetanilide. Thus, it appears that diphenylamine is at least partially metabolized to N-hydroxydiphenylamine. The N-hydroxydiphenylamine is subject to *in vivo* rearrangement to 4-hydroxydiphenylamine before excretion.

2-Nitrodiphenylamine is expected to undergo similar biotransformations. The *in vivo* formation of N-hydroxyl-2-nitrodiphenylamine is indicated by a study by American Cyanamid (1978). In this study, blood samples taken from rats 4 hours following oral administration of 3.07 g/kg of 2-nitrodiphenylamine showed 9.45% methemoglobin. The formation of 4'-hydroxyl and 4,4'-dihydroxyl-2-nitrodiphenylamine and their glucuronide conjugates would also be expected.

Although there are no data on the elimination of 2-nitrodiphenylamine from the mammalian body, its removal is expected to be rapid if the analogy to diphenylamine is valid. Alexander *et al.* (1965) found that 75% of an intraperitoneal dose of diphenylamine was excreted in the urine of rats in 48 hours. When administered intravenously, 25% of the dose was excreted in the bile after 6 hours.

The report by Alexander *et al.* (1965) on the metabolism of diphenylamine included all of the experimental details needed for corroboration of the results. Presentations made in this manner speak well for their validity. One small criticism is that no mention was made of the possible toxic effects of the metabolites discovered in the urine and bile.

2. Effects of Human Exposure

No epidemiological or occupational exposure data on 2-nitrodiphenylamine are available. American Cyanamid (1976b) recommends good personal hygiene, eye protection, good ventilation and possibly the use of a respirator for protection of workers exposed to 2-nitrodiphenylamine. No effects are expected from over-exposure.

3. Effects on Experimental Animals

a. Acute Toxicity

Only limited toxicological studies with 2-nitrodiphenylamine have been conducted. The oral LD50 of 2-nitrodiphenylamine for rats is 6.15 g/kg (American Cyanamid, 1976b). A 24-hour LD50 for skin contact in rabbits was determined to be >10 g/kg. American Cyanamid (1976b) did not observe any irritation when 2-nitrodiphenylamine was applied to rabbit eyes or skin. These data indicate that 2-nitrodiphenylamine has a low toxicity when administered in an acute dose.

b. Subacute Toxicity

When rats were administered an oral dose of 3.07 g/kg (1/2 LD50), the methemoglobin level in the blood was 9.45% in 4 hours (American Cyanamid, 1978).

c. Chronic Toxicity, Carcinogenicity and Behavior-Symptomology

No chronic studies have been performed with 2-nitrodiphenylamine. However, a large controlled study of the chronic effects of diphenylamine has been reported. It is expected that the chronic effects of 2-nitrodiphenylamine will be similar to those observed for diphenylamine. In this study, Thomas *et al.* (1967) examined growth inhibition, hematological, pathological, and urological effects of diphenylamine on weanling male and female albino rats. Groups of 20 male and 20 female rats were fed diets containing 0.0, 0.001, 0.01, 0.1, 0.5 and 1.0% diphenylamine for two years. The minimum purity of the diphenylamine used was 99.9%. Autopsies of the survivors were begun on the 734th day.

Growth curves of males and females up to the 240th day showed significant ($p<0.01$) weight arrest at the two highest levels, 0.5% and 1.0%. In addition, there was significant ($p<0.01$) weight arrest for females at the 0.1% level. Food consumption was also monitored for these

animals for the same period. Intake of food was significantly lower at the 0.5 and 1.0% levels than at lesser concentrations. It is reasonable to assume that growth arrests at these levels were caused, to a considerable degree, by reduced food intake. However, the food consumption of females receiving the 0.1% concentration was not significantly less than that of the controls. For this group, it appears that growth arrest was an effect of diphenylamine alone, not accompanied by inanition.

The amount of hemoglobin and the number of erythrocytes in rats receiving 1.0% diphenylamine in their diets was not much less than the controls by the 463rd day (Thomas *et al.*, 1967). Leukocyte counts were all within normal limits, and in addition no shifts in differential percentages occurred.

Urine samples were collected from male rats receiving the control, 0.1% and 0.5% diphenylamine diet. For each group of 5 rats, samples were collected for sixteen hour intervals on three successive days. The urine was analyzed to determine whether diphenylamine alters the amount of acid precipitable protein present (Thomas *et al.*, 1967). Normal rat urine contains some protein, but no increase in proteinuria (above control levels) was found at up to 253 days of the diphenylamine diet.

Most rats had begun to show senile changes before reaching the age of two years (Thomas *et al.*, 1967). Those rats visibly ill between the 640th day and the end of two years, and those surviving to the 734th day were sacrificed and autopsied. Lesions referable to diphenylamine toxicity were limited to the urinary tract, consisting of cystic dilation of the renal tubules and accompanying interstitial inflammation (Thomas *et al.*, 1967). Irregular dilated kidney tubules were sometimes filled with a proteinaceous fluid, and at other times with masses of iron-positive pigment, derived from blood. Sometimes these masses accumulated in the renal pelvis or bladder as non-mineralized concrements. Their presence was accompanied by mild epithelial hyperplasia or squamous metaplasia of the epithelial linings of these organs. Renal cystic changes were invariably accompanied by an accumulation of interstitial lymphocytes and subsequent scarring. Often, neutrophils collected in pigment-filled cysts. Severe inflammation was encountered only in instances of the most marked cystic changes.

The breakpoint for occurrence of chronic nephritis was at dose levels of diphenylamine between 0.1% and 0.5%. Cystic changes began to appear at the 0.1% level. At dosage levels of 0.01% and lower, lesions did not deviate from normal. Although both benign and malignant tumors were found, their incidence was not related to levels of diphenylamine in the diet (Thomas *et al.*, 1967).

The possible reproductive effects of diets containing 0.1, 0.25, and 0.5% diphenylamine were examined using 12 female and three male rats for each group. The animals began receiving the assigned diets when they were five weeks old. When they were 100 days old, they were mated, 4 females and 1 male to a cage. Then once a week, for three weeks, the males

were rotated among their three groups of females. After this time, the males were removed and the females were placed in individual cages. After all of the litters had been weaned, the parents were remated. In addition, offspring from the first mating were mated once in the same manner to examine the effects of diphenylamine on the second generation (Thomas *et al.*, 1967). Results indicated that generally litter size decreased with increasing diphenylamine concentrations. In fact, litter size decreased significantly ($p<0.05$) from the first to the second mating. For the second generation, the only significant ($p<0.01$) decrease in litter size was for the 0.1% dietary level. No histopathological or behavioral information was reported for any of these animals. It must also be recalled that the feeding of 0.5% diphenylamine was accompanied by inanition. Decreased food consumption by pregnant mothers could partially account for decreased litter sizes.

d. Teratogenicity and Mutagenicity

No studies on the teratogenicity or mutagenicity of 2-nitrodiphenylamine were reported in the literature.

E. Environmental Effects

1. Entry into the Environment

The only information available on the entry of 2-nitrodiphenylamine into the environment and its behavior in water and soil is from studies conducted on the Radford AAP effluents and the New River. Radford AAP uses 2-nitrodiphenylamine as a stabilizer in propellant formulation. The amount of 2-nitrodiphenylamine discharged into the environment from propellant manufacturing operations was estimated based on amounts of 2-nitrodiphenylamine used and known overall losses from production operations (Kitchens *et al.*, 1978).

Approximately 1-6% of the double base propellants produced are lost during processing operations (Smith and Dickenson, 1972). This percentage could account for 60-130 lb/month of 2-nitrodiphenylamine in the effluents at Radford AAP. In addition, 3-5% of the 2-nitrodiphenylamine handled may be lost during preparation operations. Thus, the total amount of 2-nitrodiphenylamine in waste streams may be 240-670 lb/month. This figure is also applicable to full mobilization operations.

2-Nitrodiphenylamine is only sparingly soluble in water. Thus, most of the lost material may be recovered as solids in the effluent stream filters. The amount actually reaching the New River as a solute in effluent streams is estimated at 1/3 to 1/2 the total lost, or 80 to 200 lb/month. The 2-nitrodiphenylamine is found in the effluent from the nitroglycerin area #2 (Outfall #22) and the solvent recovery area (Outfall #5). These areas are shown on the map of Radford AAP presented in Figure IV-4. Based on full mobilization loss estimates, 200 lb/month (6.7 lb/day), the concentrations of 2-nitrodiphenylamine that could occur in the New River at different flow rates and mixing percentages are shown in Table IV-3.

Table IV-3. 2-Nitrodiphenylamine Levels (ppm) in the New River at Full Mobilization

Degree of Mixing	Low Flow (620 mgd)	Average Flow (2,380 mgd)
1%	0.22	0.03
10%	0.022	0.003
100%	0.0022	0.0033

Weitzel *et al.* (1976) measured the concentration of 2-nitrodiphenylamine in the effluent from the nitroglycerin area #2 at Radford AAP (Outfall #22). The levels of 2-nitrodiphenylamine in the effluent ranged from 1-14 ppm with a mean of 3.5 ppm. The water flow rate from Outfall #22 at the time of sampling by Weitzel *et al.* (1976) was 3 liter/min. Thus, an average of only

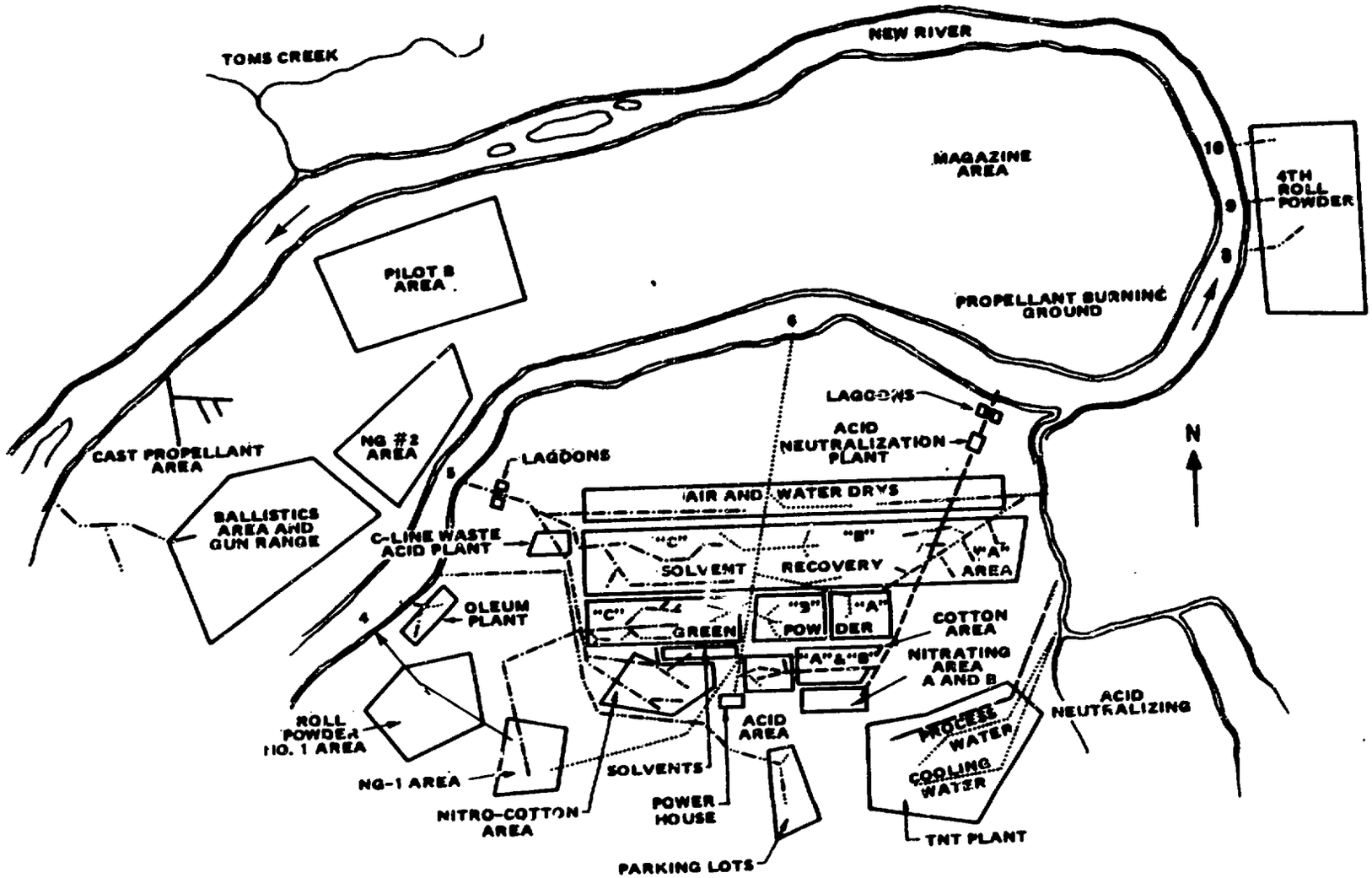


Figure IV-4. Map of Radford Army Ammunition Plant.

5.5 g/day (assuming the lines were running only 8 hr/day) were entering the New River during the sampling periods in May and October, 1975. The discrepancy between the 2-nitrodiphenylamine concentrations measured in Outfall #22 and the estimated full mobilization losses of 2-nitrodiphenylamine can be reconciled by the following facts:

- Outfall #22 is not the only source of 2-nitrodiphenylamine entering the New River from propellant production at Radford AAP.
- Weitzel *et al.* (1976) had difficulty analyzing low levels of 2-nitrodiphenylamine.
- During the 1975 sampling periods, Radford was producing propellants requiring 2-nitrodiphenylamine at less than 50% of full mobilization levels.

2. Behavior in Soil and Water

a. Transportation and Accumulation

The pH of the wastewater of Outfall #22 is near 8 and does not differ substantially from the pH of the New River. Therefore, 2-nitrodiphenylamine should not precipitate from the wastewater stream when it enters the New River. However, large increases in the flow from Outfall #22 could wash out particles of 2-nitrodiphenylamine.

Weitzel *et al.* (1976) found 2-nitrodiphenylamine in sediment samples in the New River below outfall #22. The levels of 2-nitrodiphenylamine in the sediment ranged from 0.5 to 12.2 ppm with a mean of 1.5 ppm. The levels reported were suspected to be lower than actual due to losses in the analytical procedure.

b. Degradation

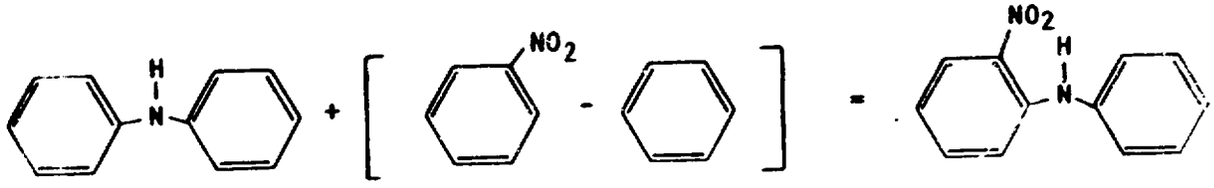
The most likely means of non-biological degradation of 2-nitrodiphenylamine is through the photodegradation mechanisms discussed in Section B.2.b. However, due to the association of 2-nitrodiphenylamine with the sediment, photodegradation mechanisms in the New River are expected to be negligible.

3. Biodegradation and Bioconcentration

a. Bioconcentration

No experimental data are available on the bioconcentration or the octanol-water partition coefficient (P) of 2-nitrodiphenylamine. However, experimental data on P for diphenylamine is available. The octanol-water partition coefficient for 2-nitrodiphenylamine was calculated by the method shown in Table IV-4. An estimation on the bioconcentration factor (BCF) of 2-nitrodiphenylamine in aquatic organisms was calculated by the following formula (Federal Register, 1979).

Table IV-4. Calculation of Octanol-Water Partition Coefficient (P) for 2-Nitrodiphenylamine



log P for Diphenylamine	+	log P for bracketed chemicals*	=	calculated log P for 2-nitrodiphenylamine
3.35	+	[1.85 - 2.13]	=	3.07

*log P values and technique from Leo *et al.* (1971) and Fujita *et al.* (1964).

$$\log BCF = 0.76 \log P - 0.23$$

log P for 2-nitrodiphenylamine was calculated to be 3.07,

therefore:

$$\log BCF = 0.76\ (3.07) - 0.23 = 2.10$$

$$BCF = 127$$

The BCF value of 127 indicates that 2-nitrodiphenylamine will be concentrated to some extent in aquatic organisms.

b. Microbial Degradation

2-Nitrodiphenylamine is capable of supporting the growth of a mixed culture of microorganisms when it is present as the sole carbon source (Kessick *et al.*, 1978). Microbial metabolism of 2-nitrodiphenylamine could lead to a variety of intermediates and products including 2-aminodiphenylamine, 4-hydroxy and 4,4'-dihydroxy-2-nitrodiphenylamine, N-hydroxy-2-nitrodiphenylamine and breakdown products thereof. However, there have been no studies to substantiate any microbial degradation pathways or degradation rates.

4. Effects on Animals

a. Mammals

The effects of 2-nitrodiphenylamine on experimental animals were presented in Section D.3. No other information on the interaction of 2-nitrodiphenylamine with mammals was found in the literature.

b. Birds

No information is available on the effects of 2-nitrodiphenylamine on birds.

c. Fish

The acute toxicity to fish of compounds similar to and potential degradation products of 2-nitrodiphenylamine are presented in Table IV-5. These data indicate that these compounds are acutely toxic in the low ppm range. It is likely that 2-nitrodiphenylamine has a similar acute toxicity.

The BCF of 2-nitrodiphenylamine was estimated at 127. Although this value is not high, 2-nitrodiphenylamine could bioaccumulate through the food chain and thus present a danger to higher organisms.

Table IV-5. Acute Toxic Effects to Fish of Compounds Similar to 2-Nitrodiphenylamine

Organism	Compound	Method of Toxicant Delivery	pH	Hardness (ppm as $CaCO_3$)	Temperature, °C	Level (in ppm)	Effect	Reference
Sea lamprey *Petromyzon marinus*	2-nitro-aniline	static	7.5-8.2	--	12	5.0	no effect	Applegate *et al.*, 1957)
Squawfish (*Ptychocheilus oregonensis*)	2,4-dinitro diphenylamine	static	7.6	0-17	12	10	death 4-7 hr	MacPhee and Ruelle, 1969
Chinook Salmon (*Oncorhynchus tshawytscha*)	"	static	7.6	0-17	12	10	death 2-3 hr	"
Coho Salmon (*O. kisutch*)	"	static	7.6	0-17	12	10	death 4-7 hr	"
Bluegill Sunfish (*Lepomis macrochirus*)	diphenylamine	static	--	--	21	1.18	96 hr LC50	Hartley, 1977
Squawfish (*P. oregonensis*)	2-amino phenyl-benzene	static	7.6	0-17	12	10	loss of equilibrium 0-½ hr	MacPhee and Ruelle, 1969
Chinook Salmon (*O. tshawytscha*)	"	static	7.6	0-17	12	10	"	"
Coho Salmon (*O. kisutch*)	"	static	7.6	0-17	12	10	loss of equilibrium 0-2hr	"

-- = not specified

d. Amphibians

No information on the effects of 2-nitrodiphenylamine to amphibians was found in the literature.

e. Invertebrates

No information was found in the literature on the toxicity of 2-nitrodiphenylamine to invertebrates. However, the ostracod, *Cyclocypris* sp., had a 48-hour LC50 of 2.53 ppm to diphenylamine (Hartley, 1977). The acute toxicity of 2-nitrodiphenylamine to this invertebrate should also be in the low ppm range.

f. Microorganisms

Only one very limited study on the microbial toxicity and biodegradation of 2-nitrodiphenylamine was reported in the literature (Kessick *et al.*, 1978). 2-Nitrodiphenylamine was found to be non-toxic to microorganisms. They also found 2-nitrodiphenylamine capable of supporting the growth of a mixed culture of microorganisms when it was present as the sole carbon source. However, no studies have been conducted to substantiate any microbial degradation pathways.

5. Effects on Plants

a. Phytotoxicity

No information is available on the toxicity of 2-nitrodiphenylamine on plants. Hartley (1977) studied the toxicity of diphenylamine on the blue, green algae, *Microcystis aeruginosa*. Diphenylamine concentrations of 0.01, 0.1 and 1.0 ppm reduced the cell density counts to 99.4, 81.6 and 61.9 percent of the controls respectively after 7 days of exposure. Diphenylamine concentrations of 5.0 ppm were lethal to the algae in 7 days.

b. Bioaccumulation

No information was found on the bioaccumulation of 2-nitrodiphenylamine by plants.

c. Degradation

No information was found on the degradation of 2-nitrodiphenylamine by plants.

F. Standards and Regulations

There are no specific United States' standards or regulations for 2-nitrodiphenylamine. This compound is listed in EPA's "Toxic Substances Control Act List of Candidate Chemical Substances;" however, EPA does not have any plans for further studies on this compound in the near future.

G. Evaluation and Comments

Only a limited amount of data was available on the toxicological and environmental properties of 2-nitrodiphenylamine. Analysis of these data indicates that 2-nitrodiphenylamine has a low mammalian toxicity. The mammalian body is capable of efficiently bio[illegible]nsforming this chemical and eliminating it. However, compounds similar t[illegible]nd probably 2-nitrodiphenylamine are toxic to aquatic organisms in low p[illegible] levels. One very precursory study indicates that 2-nitrodiphenylamine is non-toxic to [illegible]icroorganisms and can be utilized as the sole carbon source. However, the [illegible]gradation products and the environmental fate of 2-nitrodiphenylamine can only be speculated.

After evaluation of the limited information available on the toxicological and environmental properties of 2-nitrodiphenylamine, the following studies are recommended in order to further assess the environmental hazards from this compound.

1. Additional studies on the physical-chemical properties of 2-nitrodiphenylamine should be undertaken. As a minimum, the solubility of this compound in water and its octanol-water partition coefficient should be determined.

2. A field program should be initiated to determine the amount of 2-nitrodiphenylamine dischcarged into the New River as the result of propellant manufacturing at Radford AAP. Samples should be taken of the effluents from the nitroglycerin manufacture and the solvent recovery effluent from the propellant formulation area. The samples should be taken on a bimonthly or monthly basis for a period of 6 to 12 months. The 2-nitrodiphenylamine analyses should be correlated with the amount of the chemical used and the propellants being made on each sampling day. Sediment samples down river from the outfalls and local vegetation and aquatic life should also be analyzed for 2-nitrodiphenylamine concentration or accumulation.

3. A microbial degradation study on 2-nitrodiphenylamine should be undertaken. This study should be conducted in two phases. The first phase sould study the ability of New River fauna to degrade 2-nitrodiphenylamine. Organisms thriving in the New River and sediment near the outfalls containing 2-nitrodiphenylamine should be collected and cultured. Then the ability of these organisms, individually and in combination, to concentrate and degrade radio-labelled 2-nitrodiphenylamine should be determined in the laboratory. Degradation or biotransformation products should be identified. The toxicity and fate of these degradation products should also be studies.

In the second phase of the study, the ability of an activated sludge biological treatment facility to effectively degrade 2-nitrodiphenylamine to non-toxic products should be investigated.

4. Data on the toxicity of 2-nitrodiphenylamine to fish is nonexistent. The toxicity of similar compounds indicates that 2-nitrodiphenylamine should be acutely toxic to fish at the low ppm levels. Therefore, 96 hour acute static toxicity tests and 30 chronic toxicity tests should be conducted. These studies should be conducted on fish, invertebrates and algae native to the New River and in water of similar characteristics to the New River.

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PROBLEM DEFINITION STUDY ON
ETHYL CENTRALITE

SUMMARY

Ethyl centralite is used as a stabilizer in solid propellant formulations. Currently, only Radford AAP is using this compound. Most of the ethyl centralite is imported from Europe, however, Van De Marck Chemical Co. is now making ethyl centralite under contract to the Army.

In acute exposure, ethyl centralite has a low toxicity to mammals by the oral, inhalation or cutaneous routes. During chronic exposure to this chemical, only subtle biochemical changes are observed. The evidence to date suggests that this compound is not mutagenic or carcinogenic. There is no information on the teratogenic potential of ethyl centralite.

The environmental fate of ethyl centralite is not known. It is toxic to aquatic organisms in the low ppm levels. From the data available, it appears that ethyl centralite is persistent in the environment and probably bioconcentrates in aquatic organisms.

The following studies are recommended based on the potential environmental hazards of ethyl centralite.

- Sampling and analysis of Radford AAP effluents, the New River, sediment and biota
- Further studies on the physical and chemical properties of this compound
- Microbial degradation studies
- Assessment of the ability of the proposed treatment facilities at Radford AAP to efficiently remove ethyl centralite from the effluent

FOREWORD

A. Study Goals

This report presents the results of an evaluation of the available information on the toxicological and environmental hazards of ethyl centralite. Ethyl centralite is used almost exclusively as a stabilizer in propellant formulations. This compound enters the environment in the wastewater generated during the blending of propellant ingredients at Army Ammunition Plants. The wastewater generated at Army propellant manufacturing facilities is the major source of entry of ethyl centralite into the environment in the United States. The evaluation of the toxicological and environmental hazards of ethyl centralite was undertaken in order to aid the Army in identification of research needs and in recommendation of effluent criteria.

B. Study Methodology

The methodology utilized to gather information for this report included a detailed search of the literature and numerous personal contacts. During the literature search, the following sources were reviewed for pertinent information on ethyl centralite:

- Chemical Abstracts	1940 - present
- Biological Abstracts	1950 - present
- Excerpta Medica	1950 - present
- TOXLINE	1965 - present
- National Technical Information Service	1964 - present
- Defense Documentation Center	1958 - present
- COMPENDEX	1970 - present

Personal contacts were made with U.S. and foreign past and present manufacturers, Army Ammunition Plant personnel, and Army and civilian researchers.

1. Contacts with U.S. Manufacturers

   - Mr. Darrell Cardy, Cordova Chemical Co., Sept. 27, 1978. Mr. Cardy said that Beechum Chemical Company made ethyl centralite. However, Beechum was bought out by Cordova Chemical Co. Cordova does not plan to make ethyl centralite in the future. They had no toxicological or environmental data.

   - Dr. Wan, Mineral Corp., Oct. 5, 1978. Dr. Wan said that Mineral bid on a contract to make ethyl centralite and lost it to Van De Marck Chemical Co. However, he had two references for toxicological studies on ethyl centralite.

- Mr. Norm Matthews, Van De Marck Chemical Co., Oct. 6, 1978. Van de Marck Co. is currently making ethyl centralite. Mr. Matthews said they treat ethyl centlite as a dangerous industrial chemical. They had no toxicological information on the compound.

- Mr. Frank Dougherty, Pennwalt Corp., Sept. 13, 1978. Mr. Dougherty was contacted but had no information on ethyl centralite.

- Mr. Mortensen, Pennwalt Corp., Sept. 25, 1978. Mr. Mortensen did not have any toxicological information on ethyl centralite.

2. Contacts with Foreign Manufacturers

Three foreign manufacturers of ethyl centralite were listed in the 1978 Directory of Chemical Producers in Western Europe:

GERMANY, FED. REP. OF
Bayer AG
Chemische Werke Lowi GmbH

UNITED KINGDOM
Ciba-Geigy (UK) Ltd.
Division not specified

These companies were contacted by Telex in October, 1978. Two companies responded to the Telex; one had no information. Chemische Werke Lowi supplied safety data sheets and toxicological information.

3. Radford AAP Personnel

The following Radford AAP personnel were contacted Sept. 28, 1978:

Mr. John Horvath
Mr. Tom Grady
Mr. Ted Topper

None of the Radford AAP personnel had any toxicological data on ethyl centralite.

4. Other Sources

Dr. Jay Abercrombie of the U.S. Army Chemical Systems Laboratory, Aberdeen Proving Ground, Md., was contacted Sept. 17, 1978. He had no information on ethyl centralite.

Mr. J. Gareth Pearson, of USAMBRDL, Fort Detrick, Md., was visited in September, 1978. No aquatic data were available.

The Department of Transportation was contacted in October, 1978, for any toxicological information on ethyl centralite. No specific information on the compound was available.

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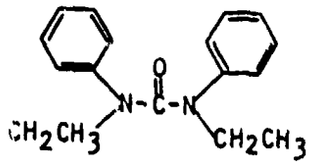
V. ETHYL CENTRALITE

A. Alternate Names

Ethyl centralite is a urea derivative possessing the following structure:



This compound is prepared by the reaction of N-ethylaniline with phosgene (Cordova Chemical Co., 1977). The primary use of ethyl centralite is as a propellant stabilizer.

Ethyl Centralite has a molecular formula of $C_{17}H_{20}N_2O$ and a corresponding molecular weight of 208.36. Alternative names for ethyl centralite are listed below:

CAS Registry No.:	85-98-3
CA Name (9 CI):	Urea, N,N'-diethyl-N,N'-diphenyl-
CA Name (8 CI):	Carbanilide, N,N'-diethyl-
Wiswesser Line Notation:	2NR&VN2&R
Synonyms:	Carbomite; Centralite; Centralite 1; 1,3-diethyl-1,3-diphenylurea; sym-diethyldiphenylurea

B. Physical and Chemical Properties

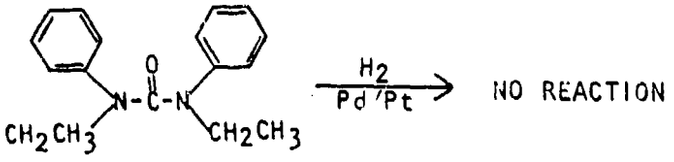
1. Physical Properties

The physical properties of ethyl centralite are presented in Table V-1. The infrared, ultraviolet and NMR spectra are shown in Figures V-1 through V-3, respectively.

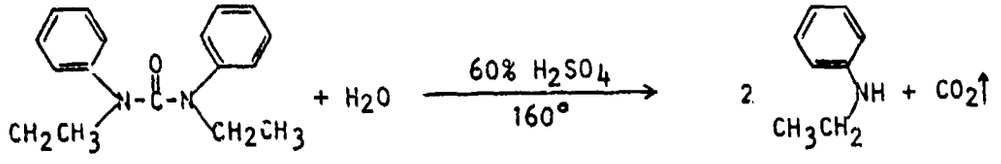
2. Chemical Properties

a. General Chemistry

Ethyl centralite is very unreactive at the amide linkage and carbonyl bond due to conjugation with the benzene rings. An attempt to reduce ethyl centralite was unsuccessful (Roy, 1969).



Basic hydrolysis of ethyl centralite does not occur, and acid hydrolysis proceeds only with great difficulty. In an 1:1 ethanol-water solution containing 15% HCl, ethyl centralite remained unreacted after 113 hours. Only by heating to 160°C in 60% sulfuric acid for 3 hours could complete hydrolysis be obtained. Hydrolysis leads to N-ethylaniline and carbon dioxide (Roy, 1968).



In contrast to the limited reactivity of the carbonyl, the benzene rings are very reactive to electrophilic substitution. This electrophilic reactivity is the basis for the use of ethyl centralite in propellants. In the propellants, the ethyl centralite reacts with the nitric acid species formed by breakdown of the nitrate explosives.

Ethyl centralite has been nitrated with nitric acid and sulfuric acid in acetic acid solvent. Complex mixtures of the possible di- and trinitro-derivatives along with traces of mono-, di-, and trinitro-N-ethylanilines are observed (Roy, 1968).

Table V-1. Physical Properties of Ethyl Centralite*

Physical Form @ 20°C:	Crystalline solid
Color:	White
Odor:	Peppery
Melting Point:	72-73°C
Boiling Point:	326°C
Vapor Pressure @ 20°C:	No data available
Flash Point:	302°C
Solubility:	Water - 80 mg/l Soluble in most organic solvents
Octanol-Water Partition Coefficient:	No experimental data available; calculated coefficient = 4.37-5.88
X-ray Diffraction Spacings and Intensities:	8.40(m-), 7.69(vs), 6.24(m), 5.29(f), 4.92(s), 4.66(s), 4.20(f), 4.09(m+), 3.78(m+), 3.65(m-), 3.42(f), 3.18(m), 2.98(f), 2.58(f+), 2.52(f), 2.46(vf), 2.39(vf), 2.33(f), 2.09(f-), 1.83(f-), (f = faint, m = medium, s = strong, v = very).

*Windholz, 1976; Sax, 1975; Hawley, 1977; Korolev *et al.*, 1976; Soldate and Noyes, 1947.

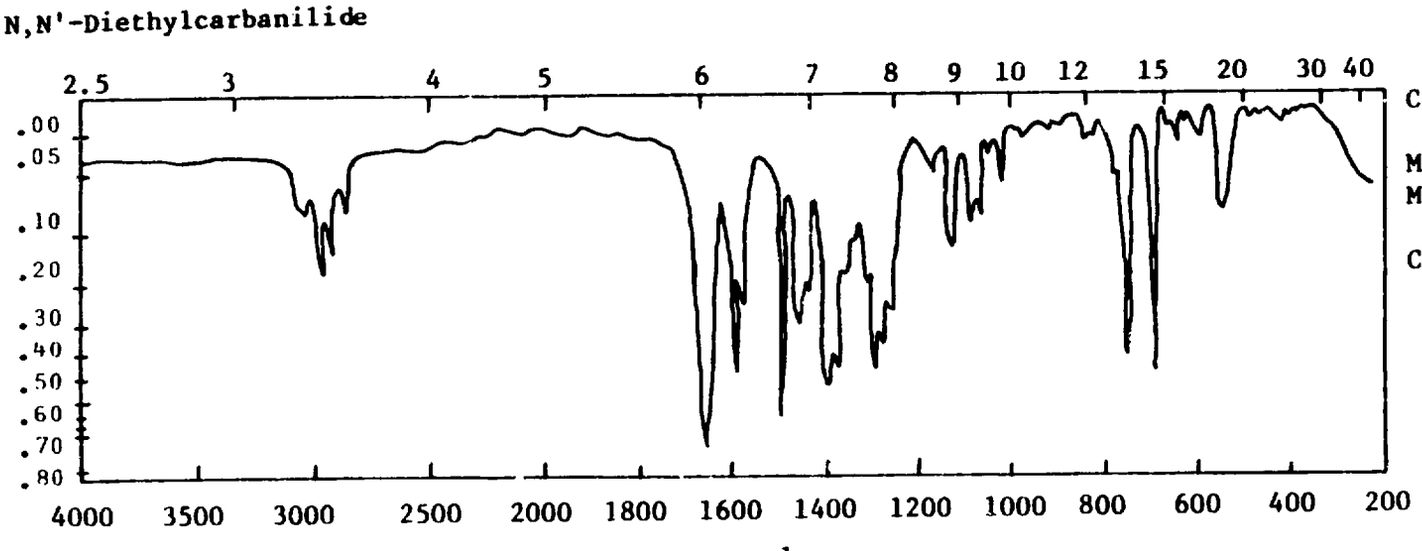


Figure V-1. Infrared Spectrum of Ethyl Centralite
(©Sadtler Research Laboratories, Inc., 1969a)

1,3-Diethylcarbanilide

952 UV

Conc. g/L A- 0.01
Slit 0.3 mm
λ Max. 247 mμ
Solvent Methanol
Cell 1 cm

Mol. Form. $C_{17}H_{20}N_2O$
Mol Wt. 268.35 M.P. 72-73°C
Source The Matheson Company, Inc. IR3219

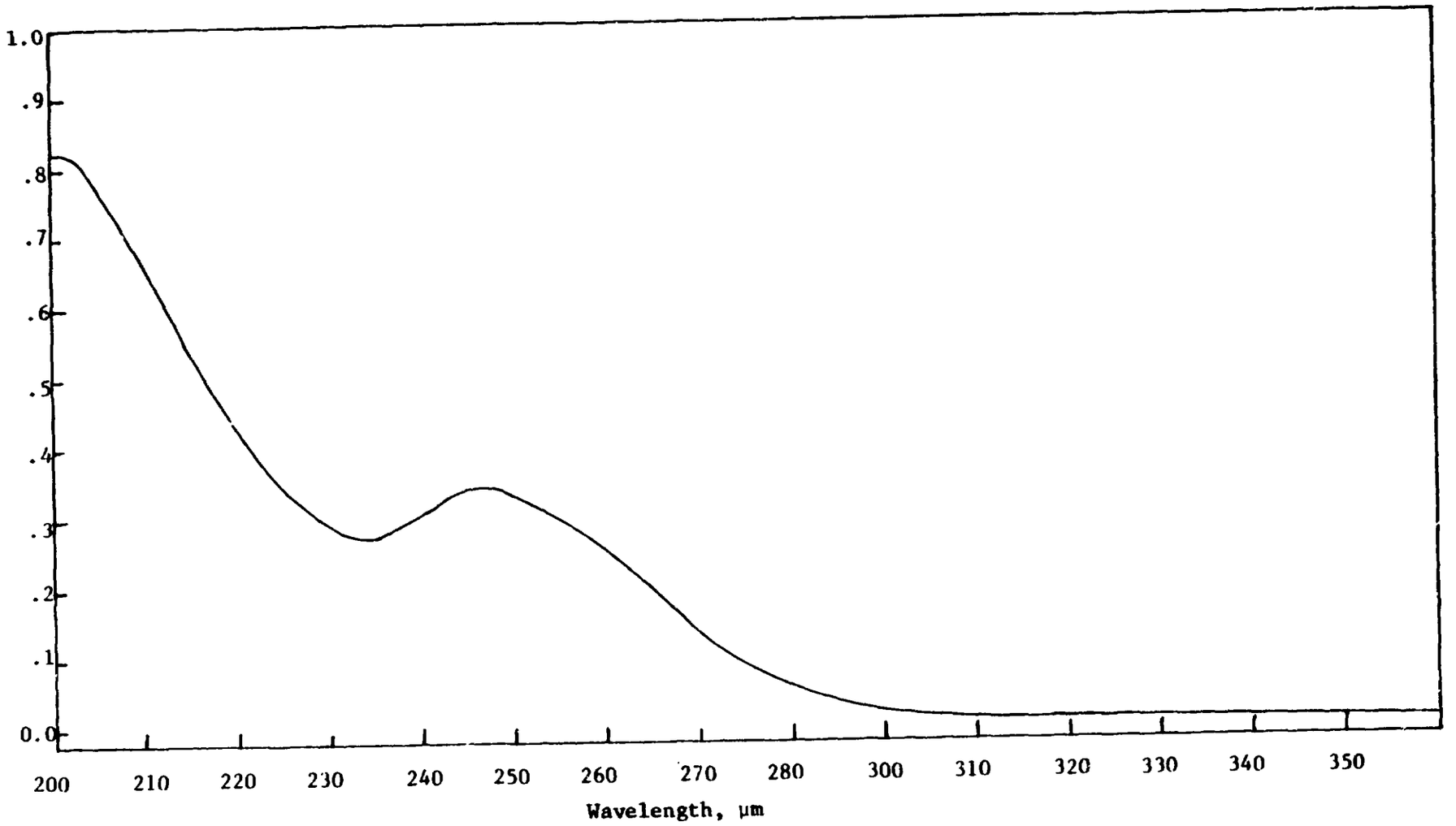
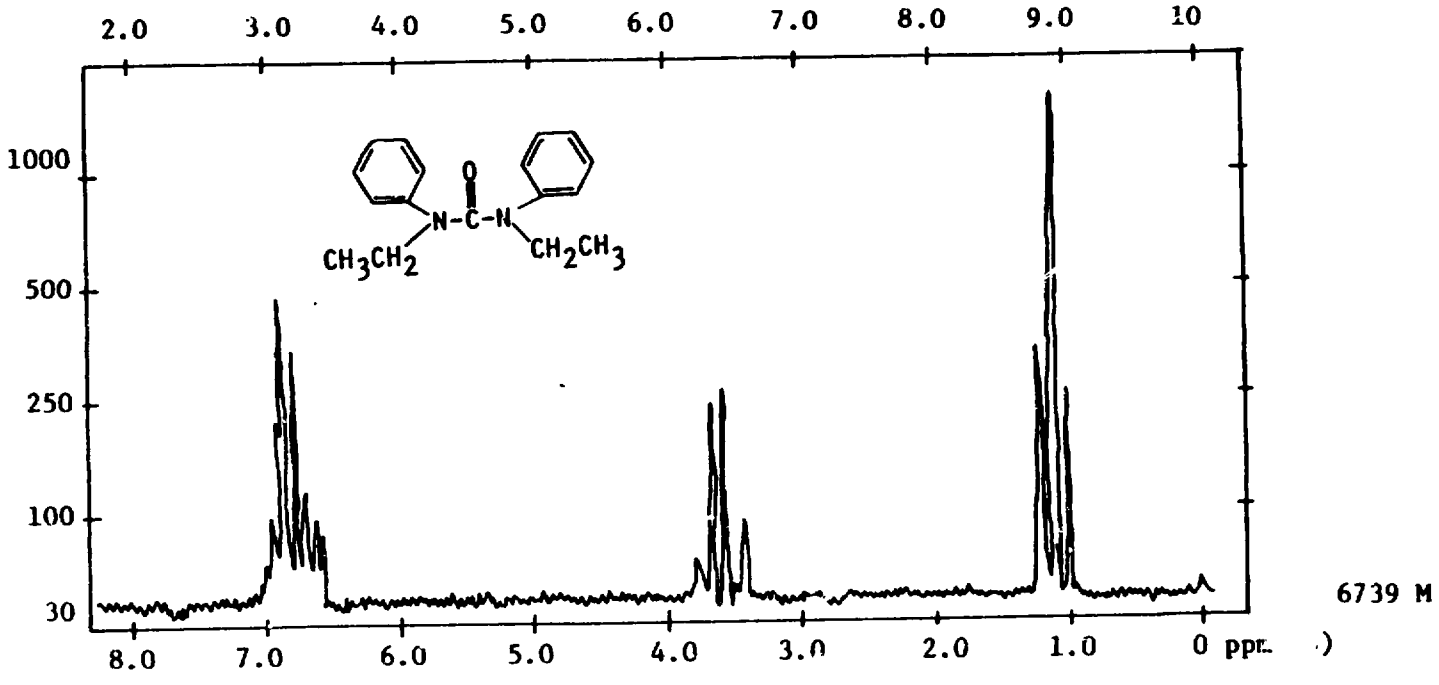


Figure V-2. Ultraviolet Spectrum of Ethyl Centralite (© Sadtler Research Laboratories, Inc., 1969b)



IR 3219; 11751

N,N'-Diethylcarbanilide

$C_{17}H_{20}N_2O$ Mol. Wt. 268.36 M. P. 72-73°C

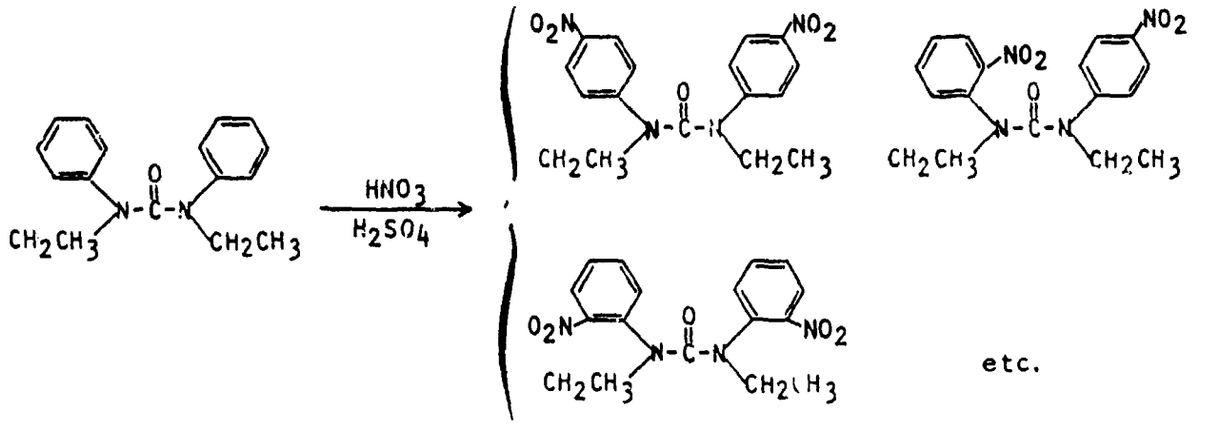
Source: The Matheson Company, Inc., East Rutherford, New Jersey

Filter bandwidth:	4	cps
Sweep time:	250	sec
Sweep width:	500	cps
Sweep offset:	-	cps
Spectrum omp:	6.3	
Integral amp:	80 (spec. amp. 3.2)	
Conc. (60mg/0.5 ml $CDCl_3$)		

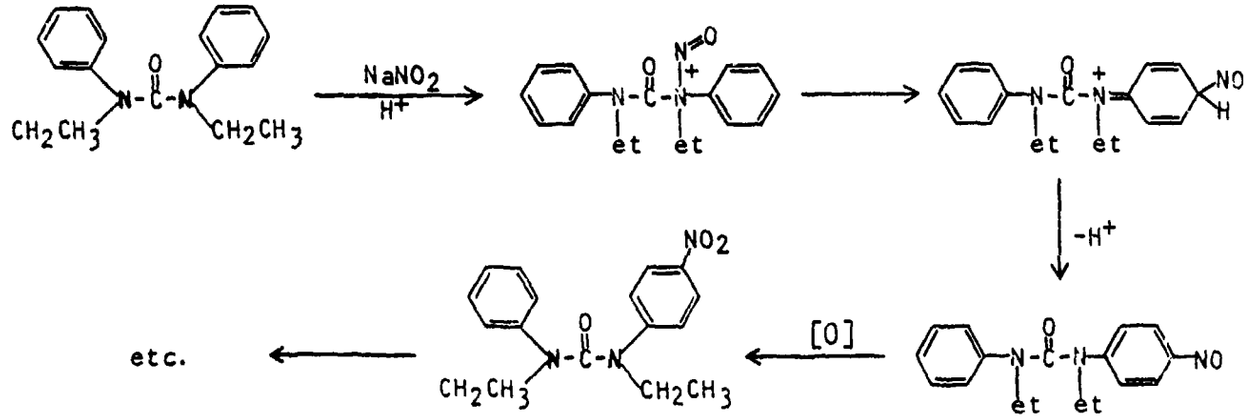
ASSIGNMENTS

a	1.11	h	
b	3.60	i	
c	6.55-7.15	j	
d		k	
e		l	
f		m	
g		n	

Figure V-3. NMR Spectrum of Ethyl Centralite (© Sadtler Research Laboratories, Inc., 1967)



When ethyl centralite was subjected to nitrous acid, similar mixtures of mono-, di-, tri-and tetranitro-derivatives are formed (Taymaz *et al.*, 1977). The most likely mechanism for this nitration involves (1) N-nitrosation followed by (2) rearrangement to the C-nitroso species and finally (3) oxidation of the nitroso species to form the corresponding nitro derivative:



This mechanism is suggested by the analogous nitrosation of dimethylaniline (Roberts and Caserio, 1965).

b. Environmental Chemistry

No reference was found to the environmental fate of ethyl centralite in the literature. Due to its extremely low solubility in water and its resistance to both acid and basic hydrolysis, ethyl centralite is probably quite persistent in the environment. Hydrolysis to N-ethylaniline and carbon dioxide, although slow, is probably the most likely the predominant degradation pathway. Photolysis would probably lead to the same products as hydrolysis since amides generally undergo cleavage at the nitrogen-carbonyl bond.

C. Monitoring and Analysis

1. Analytical Methods

Qualitative analysis for ethyl centralite has been accomplished by thin layer chromatography (TLC) on silica gel, using carbon tetrachloride/benzene/dichloroethane (Ripper, 1967), chlorobenzene/dichloro ethane (Evendijk, 1968), and two dimensional dichloroethane/petroleum ether-ethylacetate (Taymaz *et al.*, 1977; Roy, 1968) solvent systems. A similar TLC analysis has been employed as a method of determining the stability of aged propellants (Cronwall and Hildeby, 1968). Ethyl centralite has been determined quantitatively by ultraviolet absorption at 247 nm (Maurer *et al.*, 1971). Extraction of the TLC spots and ultraviolet absorption can be used to quantitate ethyl centralite. Gas chromatography has been used effectively for the analysis of ethyl centralite (Alley and Dykes, 1972; Dykes and Alley, 1974; Sopranetti and Reich, 1972; Zatkovetskii *et al.*, 1971). OV-101, OV-210 and OV-225 have been employed as stationary phases with flame ionization detection (Alley and Dykes, 1971). High performance liquid chromatography (HPLC) as well as the combination of HPLC and infrared analysis (Juhasz and Doali, 1973) have also been employed for the analysis of ethyl centralite (Doali and Juhasz, 1974). The chromatographic methods for analysis of ethyl centralite, conditions used and sensitivity of the methods are summarized in Table V-2.

2. Monitoring

The only reference to the monitoring of ethyl centralite was a Soviet study (Zatkovetskii *et al.*, 1971) of the wastewater of a chemical plant. Gas chromatography at 200-300°C on a siloxane polymer stationary phase was used in the analysis. Levels down to 5 ppm were detected. The analysis method involved the initial extraction of organics with methylene chloride, evaporation to dryness and re-dissolving the residue in dichloroethane followed by gas chromatography.

Table V-2. Chromatographic Analysis of Ethyl Centralite

Type	Reference	Stationary Phase	Mobile Phase	Detection	Conditions	Sensitivity
Column	Roy, 1968	Silicic Acid	petroleum ether, benzene, ethyl ether		-	0.5 g mixture
2-dimensional thin layer	Roy, 1968	Silica gel/Zn	$C_2H_4Cl_2$/petroleum ether ethyl acetate	UV	-	0.4 mg mixture
2-dimensional thin layer	Taymaz *et al.*, 1977	Silica gel	$C_2H_4Cl_2$/petroleum ether ethyl acetate		-	<5 mg
2-dimensional thin layer	Evendijk, 1968	Silica gel	chlorobenzene-$S_2H_4Cl_2$ (20:23)/dibutyl ether-petroleum ether (1:1)		-	15-20 mg
Thin layer	Ripper, 1967	Silica gel	CCl_4-benzene-CH_2Cl_2		-	not listed
Gas chromatography	Alley and Dykes, 1972	3.9%OV-101 or 2.5% OV-210 or 1.1% OV-225	-	FID	70-220°C	not listed
Gas chromatography	Dykes and Alley, 1974	"	-	FID	70-220°C	<5 µg
Gas chromatography	Sopranetti and Reich, 1972	2.5% OV-17, 0.5% EGS and 0.25% OS-125	-	FID	140-210°C	<0.2%
Gas chromatography	Zatkovetskii *et al.*, 1971	polymers	-	-	200-300°C	5 ppm
HPLC	Juhasz and Doali, 1973	Corasil II	cholorform, methanol, hexane (13:1:86)	UV @ 254 nm	-	15 µg
HPLC	Doali and Juhasz, 1974	Corasil II	chloroform, cyclohexone (16:84)	UV @ 254 nm	-	<100 µg

D. HEALTH EFFECTS

1. Biology

a. Absorption, Transport, Metabolism and Elimination

Ethyl centralite is absorbed into the mammalian body through the gastrointestinal tract. The amount of the ingested material which is actually absorbed is not known. Skin, eye and respiratory tract absorption also occurs but the efficiency of these processes is not known.

No information was available on the transport, metabolism or elimination of ethyl centralite.

b. Pharmacology

Only one reference to the pharmacologic interactions was found. This Russian article (Korolev *et al.*, 1976) alludes to changes in the number of erythrocytes and peroxidase activity when ethyl centralite was administered to white rats in a total oral dose greater than 110 mg/kg over 25 days. No specifics on the results were given. Over a long period of time, an oral dose of 5 mg/kg (exact time and frequency not specified) produced changes in the excretory functions of the liver and changes in peroxidase activity, ceruloplasmin and the amount of sulfhydryl groups in the blood (Korolev *et al.*, 1976). Again no specifics on the results were presented.

2. Effects of Human Exposure

No epidemiology or occupational exposure studies on ethyl centralite have been conducted. Chemische Werke Lowi (1978) recommends that respirators, gloves and goggles be used with ethyl centralite handling.

3. Effects on Experimental Animals

a. Acute Toxicity

Several studies have been conducted on the acute exposure of ethyl centralite to experimental animals. The results of these studies are summarized in Table V-3. Inspection of the table indicates that there is a discrepancy between the LD50 for oral administration to rats. The LD50 value reported by Chemische Werke Lowi (1978) is only one sixth the amount reported by the two other researchers (Korolev *et al.*, 1976); Weeks and McCreesh, 1977).

No experimental details were presented with the Chemische Werke Lowi (1978) datum. In the Weeks and McCreesh (1977) study, male Sprague-Dawley rats were fed ethyl centralite in corn oil. Observed outward signs of intoxication included tremors, lethargy, wet anus, ruffled pelt, red discharge around the eyes, and tonic convulsions at lethal doses. Korolev *et al.* (1976) observed that symptoms of acute poisoning were characterized by central nervous system disturbances and cyanosis. The low intraperitoneal acute toxicity found by Doull *et al.* (1962) indicates that the oral route of administration may not allow for efficient absorption of ethyl centralite.

Acute inhalation studies on ethyl centralite were conducted by Weeks and McCreesh (1977). Four groups of male Sprague-Dawley rats (6 each) were exposed to various concentrations of ethyl centralite vapors for 8 hours. One of the four groups was a control, exposed only to chamber air at room temperature. Two groups were exposed to nominal concentrations of 0.4 and 198 mg/l. These concentrations were obtained in chambers with flow rates of 1 l/min, in which dispersion tubes containing solid compound were heated to 50 and 100°C, respectively. For the fourth group of rats, the chamber flow rate was the same as for the two groups above, but the dispersion tube was allowed to remain at room temperature. In this case, there was no discernable loss of test material from the dispersion tube, and the nominal concentration of ethyl centralite vapor was 0 mg/l. For all cases, no toxic effects were observed during exposure and for 14 days after. In addition, all animals were sacrificed and necropsied after the 14th day. Body-weight gain and organ-to-body weight ratios of liver, kidney, lung, spleen and testes from exposed rats were not significantly different from controls. No exposure related histopathological changes were noted in the nasal turbinates, lung, heart, liver, spleen, esophagus, stomach, intestines, kidney or testes. These results show that ethyl centralite has a low volatility and should present no hazard due to inhalation of its vapors at room temperature. Also, if vapors are present, acute inhalation from a single exposure presents no hazard.

When 0.5 g of dry ethyl centralite was applied for 4 hours to either the intact or abraded skin of New Zealand white rabbits, no irritation was observed at the end of 24 and 72 hours. When the same amount of compound was applied in 1.0 ml of acetone under the same conditions, mild irritation of both the intact and abraded skin was observed at 24 and 72 hours. This irritation had disappeared 7 days after application (Weeks and McCreesh, 1977). No animals were exposed to acetone alone.

Table V-3. Acute Toxicity of Ethyl Centralite to Mammals

Animal	LD50 mg/kg	How Administered	Reference
Mice	2500	Oral	Korolev *et al.*, 1976
Rats	2750	Oral	Korolev *et al.*, 1976
Rats	420	Oral	Chemische Werke Lowi, 1978
Rats	2560 (1810-3160)[1]	Oral	Weeks and McCreesh, 1977
Mice	200	Intraperitoneal	Doull *et al.*, 1962

[1] 95% Confidence Limit

Weeks and McCreesh (1977) also tested ethyl centralite for eye irritation. Single applications of 0.5 g dry ethyl centralite to one eye of New Zealand white rabbits for 24 hours produced no opacity. However, most of the rabbits exhibited some conjuctival redness and discharge at 24 hours. After 72 hours, the eyes appeared normal. These results were interpreted to mean that working with this compound may result in moderate eye irritation.

b. Subacute Toxicity

The cumulative properties of ethyl centralite were evaluated in a subacute study conducted by Korolev *et al.* (1976). In this study, white rats (numbers unknown) were fed 22, 110 and 550 mg/kg for 25 days. These doses corresponded to 1/125, 1/25, and 1/5 of the 2750 mg/kg LD50 dose. None of these animals died during the exposure period. The following parameters were used to evaluate the toxicity of ethyl centralite: dynamic weight; erythrocyte, leukocyte and reticulocyte counts; amount of hemoglobin and methemoglobin; cholinesterase, aldolase and peroxidase activity; composition of blood protein fractions and color intensity of the urine. The animals were sacrificed at the end of 25 days, the internal organs weighed, and liver cholinesterase activity measured. At the 550 mg/kg level, a change in a number of indicator parameters was significant at the $P<0.05 - 0.01$ level. No other details of these changes were given. A change in the number of erythrocytes and in peroxidase activity was observed (no details) at the 110 mg/kg level. This dose, 1/25 LD50, was determined to be the threshold dose, the authors concluded that ethyl centralite had moderate accumulation properties. Substances with low accumulation properties have an LD50 of up to 10 times the threshold dose. An LD50 of up to 100 times the threshold dose is characteristic of a substance with moderate accumulation properties.

This study contains the only available data on the subacute effects of ethyl centralite. However, presentation of results alone without any experimental details makes the validity of those results highly suspect.

c. Chronic Toxicity

The effects of prolonged exposure to ethyl centralite were evaluated in a chronic study conducted by Korolev *et al.* (1976). White rats were fed dosages of 5, 0.5, and 0.05 mg/kg body weight over the period of the study (duration and frequency of doses not specified). In addition to those parameters

monitored in the subacute study, the following indicators were also monitored: conditioned reflex activity, ceruloplasmin, β-lipoprotein in the blood serum during fat metabolism, sulfhydryl groups, transaminase and phosphatase activity, the liver excretory function and semen. No changes were observed in these parameters in the rats receiving 0.5 and 0.05 mg/kg dosages. However, statistically significant changes in the conditioned reflex activity, liver excretory function, peroxidase activity, ceruloplasmin and sulfhydryl groups in the blood were observed in rats receiving the 5 mg/kg dosage.

Although it is commendable that a large number of parameters were monitored in this study, nothing is known of the particulars of the experiment. If indeed statistically significant changes in various parameters were observed, supportive data should have been provided. The credibility of results such as these is dubious.

d. Teratogenicity, Mutagenicity, and Carcinogenicity

Korolev *et al.* (1976) also evaluated the gonadotropic and mutagenic effect of ethyl centralite during the chronic study discussed in the preceding section. The functional state of the spermatozoa, the morphology of the seminal canal epithelium, the number of chromosomes showing reorganization in the anaphase and the absolute number of fragmented types being recombined were measured. No signs of any gonadotropic or mutagenic effects were found even at the 5 mg/kg dosage.

Although the data for part of this experiment are included, the actual details are again omitted. If these particulars had been included, the finding of no gonadotropic or mutagenic effects would have been more believable.

Weeks and McCreesh (1977) tested one strain of the yeast *Saccharomyces cerevisiae* and five strains of the bacterium *Salmonella typhimierium* to evaluate the mutagenicity of ethyl centralite. Results showed that ethyl centralite did not demonstrate mutagenic activity in any of the tests conducted.

No evidence was found in the literature to indicate any carcinogenic properties associated with ethyl centralite.

e. Behavior - Symptomology

There are no significant outward signs of chronic toxicity due to ethyl centralite exposure. Therefore, biochemical changes must be used to diagnose ethyl centralite intoxication.

E. Environmental Effects

1. Entry into the Environment

Ethyl centralite is used as a stabilizer in propellant formulations currently produced at Radford AAP. Badger, Sunflower and Indiana AAP's also use this compound when they are operational. During 1977, Radford AAP used ethyl centralite at an average rate of 31,200 lb/month (Watts, 1978). Kitchens *et al* (1978) estimated that at full mobilization Radford AAP would consume about 150,000 lb/month of ethyl centralite.

It is estimated that 1-2% of the propellants including ethyl centralite produced are lost during processing operations. Another 2-3% of the ethyl centralite handled may be lost during preparation operations (Dickenson, 1978). Thus, the total amount of ethyl centralite in waste streams amounts to 900-1600 lb/month, based upon current production rates at Radford AAP. Full mobilization losses would range from 4,500 to 7,500 lb/month.

Ethyl centralite has a solubility of 80 ppm in water. Much of the material lost is collected in solid form on effluent stream filters. This material may be collected and burned, thus never reaching the New River. However, depending upon the frequency of particulate removal from the filters, some ethyl centralite may leach out of the collected solids. It is estimated that 1/3 of the total ethyl centralite loss may ultimately appear in effluents reaching the New River. Losses of ethyl centralite from Radford AAP operations to the New River would be 300 to 530 lb/month at current levels and 1500 to 2500 lb/month at full mobilization (Kitchens *et al.*, 1978).

Based on these loss estimates, potential levels of ethyl centralite in the New River at various flow rates and degrees of mixing were calculated. These data are presented in Table V-4. At the present time, the major point of entry of ethyl centralite into the environment in the United States is from Radford AAP operations.

Table V-4. Estimated Ethyl Centralite Levels (ppm) in the New River

Degree of Mixing	Low Flow (620 MGD) Normal Use	Full Mobilization	Average Flow (2380 MGD) Normal Use	Full Mobilization
1%	.50	1.61	.12	.41
10%	.050	.16	.012	.041
100%	.005	.016	.0012	.0041

2. Behavior in Soils and Water

Ethyl centralite has a solubility of 80 ppm in water and should initially be found in the water. However, due to its high solubility in organic compounds, ethyl centralite could be adsorbed into sediments.

Ethyl centralite is resistant to acid and base hydrolysis. No information was available on the possible photodegradation of ethyl centralite. If the chemical is degraded, the resulting products will most likely be N-ethylaniline and carbon dioxide.

3. Bioconcentration and Biodegradation

a. Bioconcentration

No data are available on the bioconcentration of ethyl centralite by aquatic organisms or on the octanol-water partition coefficient (P) of this compound. Thus, in order to estimate the bioconcentration of ethyl centralite, the octanol-water partition coefficient was calculated based on data available on similar compounds.

Two possible methods for calculating the octanol-water partition coefficient (P) for ethyl centralite are presented in Table V-5. The two estimates give a log P for ethyl centralite of 4.37 - 5.88. Other variations in calculation of P for ethyl centralite gave log P values between 5.0 - 5.5. The bioconcentration factor (BCF) for ethyl centralite was estimated using the following formula (Federal Register, 1979).

$$\log BCF = 0.76 \log P - 0.23$$

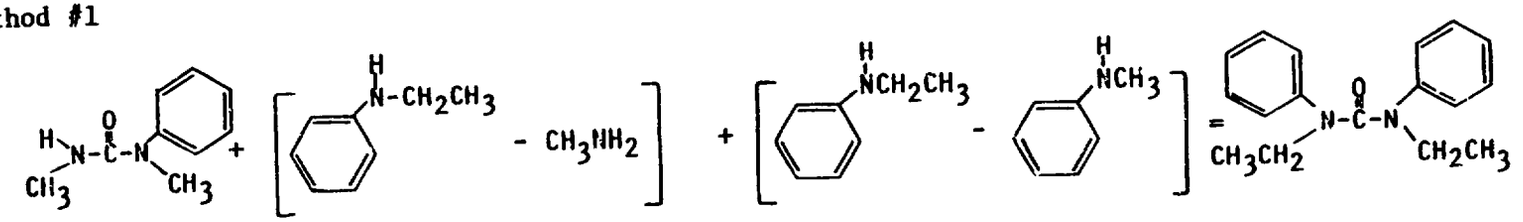
The range for the bioconcentration factor (BCF) in aquatic organisms, with an assumed 8% liquid content, would be from 1250 to 17,300. These numbers indicate that ethyl centralite is highly concentrated by aquatic organisms. Using the value of 1.0 ppb for ethyl centralite in water (See Table V-4), aquatic organisms could concentrate from the water levels between 1.2 and 17.3 ppm.

b. Biodegradation

No information is available on the biodegradation of ethyl centralite.

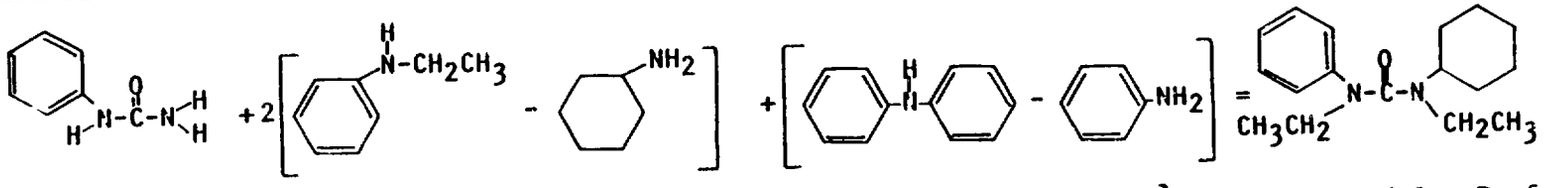
Table V-5. Calculated Octanol-Water Partition Coefficient (P) for Ethyl Centralite

Method #1



log P of 1,3-dimethylphenyl urea	+	[log P of N-ethylaniline − CH_3NH_2]	+	[log P of N-ethylaniline − log of N-methylaniline]	=	estimated log P of ethyl centralite
1.02	+	[2.26 − (−.57)]	+	(2.26 − 1.74)	=	4.37

Method #2



log P of phenyl urea	+ 2	[log P of N-ethylaniline − log P of aniline]	+	[log P of diphenylamine − log P of aniline]	=	estimated log P of ethyl centralite
.82	+	2 [2.26 − .94]	+	[3.36 − .94]	=	5.88

*log P values and technique from Leo *et al.* (1971).

4. Effects on Animals

a. Mammals

The only available information on the effects of ethyl centralite on mammals in on experimental animals. These effects were discussed in Section D.

b. Birds

No information is available on the environmental effects of ethyl centralite on birds.

c. Fish

The aquatic toxicity of ethyl centralite to fish is presented in Table V-6. Although a no effect level cannot be established, ethyl centralite was acutely toxic at levels of 10 ppm and rapidly stressed fish at levels of 5 ppm. The data indicate that ethyl centralite has a moderate to high toxicity.

d. Amphibians

No studies have been conducted on the environmental effects of ethyl centralite on amphibians.

e. Invertebrates

No data are available on the aquatic toxicity of ethyl centralite to invertebrates.

f. Microorganisms

Korolev *et al.* (1976) found that ethyl centralite levels greater than 10 ppm inhibited the growth of Saprophytic microflora and retarded the degradation of organic pollutants.

5. Effects on Plants

No information was found on the effects of ethyl centralite on plants. Limited information was obtained on the phytotoxicity of N-ethylaniline, the potential degradation product of ethyl centralite. The effects of N-ethylaniline and related compounds to algae are presented in Table V-7.

Batterton *et al.* (1978) observed that N-ethylaniline had a low toxicity to the blue-green algae, *Agmenellum quadruplicatum*, when compared to other substituted anilines.

Table V-6. Toxicity of Ethyl Centralite to Fish

Organism	Compound	Method of Toxicant Delivery	pH	Hardness (ppm as $CaCO_3$)	Temperature °C	Level (ppm)	Effect	Reference
Northern Squawfish (*Ptyenocheilus oregomensis*)	Ethyl Centralite	Static	7.2	0-17	12	10	Death in 1-3 hours	MacPhee and Ruelle, 1969
Chinook Salmon (*Oncorhynchus tshawytscha*)	" "	Static	7.2	0-17	12	10	Death in 1-3 hours	"
Caho Salmon (*O. kisutch*)	" "	Static	7.2	0-17	12	10	Death in 1-3 hours	"
Bluegill (*Lepomis microchirus*)	" "	Static	7.5-8.2	n.s.	n.s.	5.0	Stress in 1 hour	Applegate *et al.*, 1957
Rainbow Trout ((*Salmo fairdneri*)	" "	Static	7.5-8.2	n.s.	n.s.	5.0	Stress in 5 hours	"
Larval Lamprey (*Petrommon marinus*)	" "	Static	7.5-8.2	n.s.	n.s.	5.0	No deaths	"

n.s. = not specified

Table V-7. Toxicity of Potential Ethyl Centralite Degradation Products and Related Compounds to Algae

Algae	Compound	Hardness (ppm as $CaCO_3$)	Temperature °C	Dose	Effect	Reference
Blue Green (*Agmenellum quadruplicatum*)	N-ethylaniline	-	-	500 µg/plate	5% inhibition of growth	Batterton *et al.* (1978)
"	aniline	-	-	10 µg/plate	100% inhibition of growth	"
Green (*Scenedesmus quadricauda*"	aniline	16	25	8.3 ppm	toxicity threshold	Bringmann and Kuhn (1977)

F. Regulations and Standards

No effluent or industrial hygiene standards exist for ethyl centralite in the United States. The compound is not listed in the "EPA Toxic Substances Control Act Candidate List of Chemical Substances."

Korolev *et al.* (1976) suggested a maximum permissible level of ethyl centralite in water of 0.5 ppm for Russia.

G. Evaluation and Comments

Two fairly comprehensive studies have been conducted on the toxicity of ethyl centralite to mammals. These studies indicate that ethyl centralite has a low acute toxicity. Chronic intoxication results in subtle biochemical changes. Evidence indicates that ethyl centralite is not mutagenic or carcinogenic. The effects of this compound on the developing fetus have not been evaluated. Thus with proper safety equipment and personal hygiene, ethyl centralite should not be a toxic hazard to workers.

There is no information on the environmental fate of ethyl centralite. Hydrolysis could occur but only very slowly. Bioconcentration and bioaccumulation of this compound is expected but has not been confirmed. Ethyl centralite is toxic to many aquatic organisms in the low ppm levels. Thus ethyl centralite could present a significant environmental hazard to life in and surrounding the New River.

Based on the potential environment hazard of ethyl centralite, the following studies are recommended in order to fill in the information gaps on this compound.

1. Sampling and analysis of the Radford AAP effluents to determine actual ethyl centralite concentrations and correlation of the effluent levels with production. Sampling and analysis of the New River, sediment and biota to determine the environmental fate of this compound and its bioconcentration potential.

2. Further investigation of the physical/chemical properties of this compound including octanol-water partition coefficient, hydrolysis, and photochemistry.

3. Microbial degradation studies on ethyl centralite to determine the ability of the organisms in the New River to degrade this compound and the degradation products.

4. Investigation of the ability of the proposed industrial waste treatment facility at Radford AAP to effectively remove ethyl centralite from the effluents.

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ABBREVIATIONS AND SYMBOLS

$\mathring{A}$	- Angstrom
@	- At
AAP	- Army Ammunition Plant
ANOVA	- Analysis of Variance
$\sim$	- Approximately
BCF	- Bioaccumulation Factor
BODS	- Biochemical Oxygen Demand
B.P.	- Boiling Point
BTU	- British Thermal Unit
^{o}C	- Degree Centigrade
Cal	- Calorie
cm	- Centimeter
cm^{-1}	- Wavenumber
cm^{3}	- Cubic Centimeter
COC	- Cleveland Open Cup
COD	- Chemical Oxygen Demand
C_p	- Heat capacity at constant pressure
cP	- Centipoise
DOT	- Department of Transportation
ΔH_c	- Heat of Combustion
ΔH_f	- Heat of Fusion
DMSO	- Dimethylsulfoxide
ξ	- Molar extinction coefficient
ϵ	- Molar absorptivity coefficient
E^{+}	- Electrophile
^{o}F	- Degrees Farenheit
FIR	- Far Infrared
g	- Gram
gal	- Gallon
H^{+}	- Hydrogen Ion
HMX	- Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
hg	- Mercury
hr	- Hour
HPLC	- High Pressure Liquid Chromatography
$h\nu$	- Light energy
in	- Inch
i.p.	- Intraperitoneal
i.v.	- Intravenous
IR	- Infrared
J	- Joule
^{o}K	- Degrees Kelvin
kbar	- kilobar
kcal	- Kilocalorie
kg	- Kilogram
KKg	- Thousand Kilograms
kp	- Partition Coefficient
Ksp	- Solubility Product Constant
l	- Liter

λ	- Wavelength
lb	- Pound
LC20	- Concentration Required to Kill 20% of Exposed Population
LC50	- Concentration Required to Kill 50% of Exposed Population
LC100	- Concentration Required to Kill 100% of Exposed Population
LD50	- Dosage Required to Kill 50% of the Exposed Population
LDLO	- Lowest Dosage for Which an Effect is Observed
λmax	- Wavelength of Absorption Maxima
m	- Meter
m^3	- Cubic meter
μ	- Micron
m/c	- Mass to Charge Ratio
μg	- Microgram
mg	- Milligram
MGD	- Million Gallons per Day
ml	- Milliliter
mm	- Millimeter
mM	- Millimoles
mmHg	- Millimeters of Mercury
mol	- Moles
M.P.	- Melting Point
M.W.	- Molecular Weight
N	- Newton
n_D	- Refractive Index
NMR	- Nuclear Magnetic Resonance
nm	- Nanometer
π	- Pi (a constant $\approx$ 3.1416)
P_d	- Pressure in Newtons/Meter^2
P_{cv}	- Detonation Pressure @ Constant Volume
P	- Partition Coefficient
%	- Percent
pH	- Negative Log of Hydrogen Ion Concentration
ppb	- Parts per Billion
ppm	- Parts per Million
RDX	- Hexahydro-1,3,5-trinitro-1,3,5-triazine
R	- Refractive Index
SEX	- 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine
T	- Temperature ^{o}Kelvin
TLC	- Thin Layer Chromatography
TAX	- 1-acetylhexahydro-3,5,-dinitro-1,3,5-triazine
Torr	- Unit of pressure
U.V.	- Ultraviolet
vol	- Volume
wt	- Weight
yr	- Year

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